# CELLULAR DEVELOPMENT OF THE AVOCADO FROM BLOSSOM TO MATURITY

By

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# INTRODUCTION

The fruit of the avocado (Persea americana Mill.) has many characteristics of growth, development, and physiology which differ markedly from those of most deciduous and many evergreen tree fruits. For instance, the avocado fruit does not soften until removed from the tree and may be held on the tree for periods up to 6 months or more following the attainment of horticultural maturity. Another difference is that in the avocado fruit cell division continues throughout the time the fruit remains on the tree. Unlike fruits of most deciduous orchard species, in which cell division ceases a few weeks after pollination and further enlargement is only by increase in cell size, in the avocado cell division is an important factor in the increase in fruit size in the later phase of fruit development. Physiologically, the fruit exhibits an unusually high rate of metabolic activity following harvest. Its respiratory rate is approximately 2 to 3 times that of the banana, orange, apple, or other common fruits.

Growth of fruits is a problem, not only of considerable physiological interest but also of outstanding economic importance. The structure of the epidermis, for instance, has often been regarded as an important factor in determining the shelf-life of many fruits. The cuticle of the 'Golden Delicious' apple compares favorably in thickness with other apple varieties; however, the development of cracks during matura-

tion has been ascertained as the cause of its extreme susceptibility to early shrivelling in storage. Russetting, a common physiological disorder in 'Golden Delicious' apples, is considered partly the result of some anatomical peculiarities in the structure of the fruit during development. Some of the heaviest losses of thin-skinned avocado fruits result from end-spots. In end-spotted fruit the larger end gradually withers and dries, and in some cases cracking may proceed until the seed is exposed. Since keeping-quality is related to the physiology of the fruit, it is of considerable interest to investigate the anatomical and histological causes of differences in structure and to relate these to physiological phenomena, both during the development of the fruit and during its senescent life after removal from the tree.

The anatomy of the different parts of the avocado fruit has been studied on several occasions, and the growth and development of the mesocarp tissue from pollination to horticultural maturity has been investigated, but the various workers on this fruit did not study the morphogenesis of the various organs and tissues continuously from blossom to senescence.

This investigation was initiated with the hope of determining additional facts about the anatomy and histology of the fruit of the different horticultural races of avocado. These facts could then be used as a basis for an enlightened study on the physiology and storage of avocado fruit.

## LITERATURE REVIEW

### A. General Morphological and Anatomical Development of Fruits

The physiology of fruit ripening has been studied extensively, under the pressure of practical problems, and books dealing primarily with post-harvest physiology of such fruits as the apple (58), banana (27), and orange (52) have been published. On the other hand, the physical and morphological aspects of growth and development in fruits have received less attention.

The early workers who studied morphology of fruits, such as Farmer (12), Kraus (22), Winton (70), and Cummings and Schroeder (10), dealt primarily with the structure of the mature fruit and recorded but few significant structural changes in the developing fruit.

Later workers have been more concerned with external changes of the developing fruit, as well as growth in respect to the development of the component tissues of the fruit. When establishing growth curves, volume or weight of the fruit is plotted against time. Whether the volume is obtained by direct determination (15) or by calculation (54), fresh or dry weight (15), or diameter (18,33,34), there generally results a sigmoid curve, which is characteristic of the growth of plant organs and of organisms in general. The pome fruits have perhaps received the greatest attention. In the case of the apple, it has been concluded (4,6,62,68), that in the flower-bud stage, the rudimentary

apple consists mainly of a mass of meristematic cells through which vascular bundles are differentiating. During the flowering and fruit-setting stages, there is a period of rapid cell division in all parts of the ovary and associated tissues, extending for approximately 3 weeks. Cell enlargement begins after cell division has ceased and continues throughout the life of the fruit as long as it remains on the tree. This rapid cell enlargement, together with development of intercellular spaces, is responsible for the later part of fruit growth.

Fruit size at maturity was found by Smith (56) to be determined by the amount of cell multiplication and by the degree of cell enlargement; either factor might be dominant. Better-keeping varieties have longer growth periods, a smaller number of cells per unit weight of cortex, and a lower level of respiratory activity, according to Smith (57). Poorer-keeping varieties have a shorter growth period, larger number of cells per unit weight of cortex, and a higher level of respiration activity.

Except for a slightly longer cell-division period and sclereid formation in the cortex, the morphological and anatomical changes in pear fruits are essentially the same as in the apple (5,30,31,63).

The situation among drupe fruits is quite different. Instead of the characteristic sigmoid curve, 3 growing periods have been distinguished. In Period I, beginning about the time of full bloom, the fruit enlarges rapidly and the integuments of the seed develop, but the embryo remains small. During Period II, the embryo develops, but the growth of the fruit stands still. As embryo growth ceases, the fruit enters a second period of rapid enlargement extending to fruit ripen-

ing, which constitutes Period III. Fruits such as the peach (25,65), plum (26), apricot (24), sweet cherry (64), and sour cherry (66,67) have shown this type of growth pattern, which points toward a certain competition between developing embryos and fruits.

## B. Gross Morphological and Histological Development in the Avocado Fruit

### Floral Anatomy of the Avocado

The avocado flowers are small, about 12 mm across when fully open. They are perfect, hypogynous, trimerous, and are borne in cymose panicles. Study of the vascular anatomy of the flower supports Van Tieghem's floral formula of (3S + 3P + 3E + 3E' + 3E'' + 3E''' + C) (37), but the calyx and corolla are distinguishable only by position and are conveniently termed perianth lobes (39). The bases of the 2 perianth whorls are coherent, thus forming a cup upon which are inserted 4 stamen whorls of 3 members each. Reece (37) noted that "the anthers of the 2 outer whorls are introrse. Those of the third whorl are extrorse and bear glands on each side of the filament near the point of attachment on the perianth. The fourth whorl has been reduced to gland-like staminodes." The single pistil consists of 1 carpel with 1 ovule.

According to Reece (37), the vascular tissue in the pedicel is arranged in a siphonostele. A cross section through the pedicel reveals 6 areas of primary xylem standing out sharply. At higher levels towards the receptacle, the 6 perianth traces depart from the stele and pass through the cortex. The gaps left after the departure of the perianth traces close quickly but the vascular tissue of the stele "shrinks" as

it approaches the tip of the receptacle. Before the vascular tissue fades out, however, the carpellary traces depart near the tip of the axis and enter the ovary. The same worker said that the vascular supply of the ovary "consists of a dorsal trace, 2 or more lateral traces, and 2 ventral traces which are united into a single, morphologically compound vascular bundle." The dorsal and 2 lateral carpellary traces extend up through the simple style. The strong dorsal trace continues into the stigma but the lateral traces disappear in the upper portion of the style. The fused ventral trace enters the funiculus of the large, anatropous ovule and passes down the opposite side of the ovule near the dorsal part. This bundle then branches profusely, investing the integuments with a network of vascular bundles conspicuously developed in the seed coats of mature fruit.

The compound sepal and petal traces in the perianth cup divide tangentially to produce the traces of the 4 stamen whorls and radially to produce branch traces in each whorl. Each stamen has 3 traces traversing the major portion of the filament. The glands of the third whorl of stamens are supplied by 2 additional traces.

Floral Development, Sporogenesis, and Embryology.

Floral differentiation in the avocado continues over a period of months during that part of the year when growth is at a minimum. In Florida (38), differentiation begins in late October or November, but the earliest primordia of the individual flowers are not identifiable until January, only a few weeks before full bloom. The relatively short interval between flower-bud initiation and full bloom in the avocado was

confirmed by Schroeder in California (43).

The flower parts of the avocado develop in acropetal order. The last floral organ to develop is the pistil. Schroeder (44) reported that the pistil "first appears as a small, conical mass which by differential growth produces a cup-like structure, one side of which becomes higher than the other. The higher side gives rise to the style and stigma, and the lower, which grows more slowly, differentiates the ovule primordium on its inner side." The upper side of the funiculus grows more rapidly, causing the ovule to bend toward the receptacle. The integuments and the nucellus are differentiated at this time. "By continued bending the ovule eventually becomes anatropous, and the micropyle is located immediately beneath the funicular attachment" (44).

Megasporogenesis is initiated with the formation of a single hypodermal archesporial cell. This divides and gives rise to the megaspore-mother cell and the primary parietal cell. By 2 successive divisions of the megaspore-mother cell a linear row of 4 megaspores is formed. The 3 micropylar megaspores degenerate but the chalazal spore enlarges and by 3 successive nuclear divisions gives rise to an 8-nucleate megagametophyte. The mature embryo sac is elongated (44).

Schroeder (44) also reported that microsporogenesis begins with the formation of a hypodermal sporogenous cell in the young anther. In rapid succession several of the adjacent hypodermal cells become sporogenous in nature. Continued cell division gives rise to the sporogenous tissue which eventually becomes a mass of pollen-mother cells surrounded by a tapetal layer. The pollen-mother cells undergo meiosis and form tetrads which soon separate into individual spores. Mature pollen

grains are spherical with no germ pores and are covered with short, conical spines.

In an earlier study of microsporogenesis in the avocado and related species the haploid chromosome number was determined to be 12 (69).

The endosperm is a triple-fusion tissue. Whether a free nuclear stage exists was not ascertained, but Schroeder (44) observed that the development of the endosperm precedes that of the embryo. Endosperm cells have extremely large nuclei and sometimes attain a diameter of 100 microns. Reserve materials found in the endosperm consist mainly of soluble sugars. Starch, oils, or tannins were not observed.

"Development from the zygote to the multicellular proembryo takes place over a long period of time, probably many days," according to Schroeder (44). The growing proembryo remains spherical until it attains a diameter of about 0.2 to 0.3 mm when cotyledonary differentiation begins. The cotyledons differentiate further, and later the young radicle and plumule appear at the cotyledonary node together with the provascular tissue. "Differentiation in the mature embryo continues until there are two hemispherical cotyledons with vascular strands located in a plane parallel to their flat faces and close to these surfaces" (44).

G.D

Floral Biology

The flower-bud system in the avocado is derived from terminal and subterminal buds on growth of the previous season. Ordinarily the buds are mixed and contain both floral and vegetative primordia. Concurrent with shoot elongation the lateral branches or secondary shoots

again become simply or highly branched structures upon which the individual flowers are borne. The terminal portion of the main axis, from which subsequent growth in length continues, remains vegetative. Occasionally, shoot tips are found which terminate in a flower bud from which no further elongation can occur (38,41).

The avocado flower is perfect in form but not in function. Nirody (32) discovered the dichogamy in avocado. He observed that the pistil matures before the pollen is shed and that this alternate development of the essential parts of the flower is decidedly synchronous. Later, Stout (61) reported that each flower normally has 2 distinct periods of opening instead of one. At the first opening of every flower, the pistil becomes receptive but the stamens do not shed pollen. The flowers open for the second and last time the next day when the pollen is shed. A night always passes between the first and second opening. Furthermore, varieties whose first opening is in the morning (Class A) have their second opening in the afternoon of the next day. Those varieties whose first opening is in the afternoon (Class B) have the second opening the next morning. Because of this unusual dichogamy it is normally difficult for the avocado to self-pollinate. However, Stout (61), Schroeder (46), and others have found that sudden changes in temperature and cloudiness affect the daily periodicity of avocado flowers. Rainy periods and sudden drops in temperature sometimes cause overlapping of flower sets, rendering individual trees partly self-fruitful.

A number of insects are helpful in pollinating the avocado. The pollen after its transfer to the stigmas of newly opened flowers has been observed to germinate quickly (46). The pollen tube grows down-

ward through the style tissue to the ovule and egg cell. Under favorable conditions, fecundation has been estimated to take place in approximately 28 hours after pollination. Slow-growing pollen tubes may require 66 hours to reach the egg cell (46). Stimulation of cell division in the ovary wall is initiated at the time of embryo formation, and growth of the fruit then commences (47).

Anatomy of the Avocado Fruit

The gross structure and morphology of the avocado fruit were investigated by Cummings and Schroeder (10). They reported that the avocado is a berry of 1 carpel containing a single seed. The pericarp, according to these workers, consists of 3 layers: the exocarp, which comprises the skin or rind; the fleshy mesocarp, which is the edible portion of the fruit; and a thin inner layer next to the outer seed coat, the endocarp.

The exocarp is the layer that is removed when the fruit is peeled, and is made up of epidermal, sclerenchyma, and parenchyma tissues. A relatively thin cuticle forms a wax-like film over the surface of the fruit. Beneath this film is an epidermis of 1 layer and a hypodermis of 1 to 3 layers of brick-shaped cells. Beneath the epidermal and hypodermal layers, but still a part of the exocarp, lie several layers of parenchyma cells. These cells are isodiametric in form, measure approximately 40 microns in diameter, and contain chloroplasts, some oil, and tannin. Next inside this parenchymatous tissue, and limiting the exocarp on the inner surface, is an interrupted layer of sclerenchyma tissue. Cummings and Schroeder (10) showed that differ-

ences between thick-skinned and thin-skinned fruit types resulted primarily from the amount and density of these stone-cell masses. Varieties with thick rinds, such as characterize those of the Guatemalan horticultural race, have a layer of very densely packed sclerenchyma, while thin-skinned varieties of the Mexican horticultural race contain only scattered groups of loosely packed, small sclerenchyma just beneath the epidermis.

In a more detailed study of the avocado rind, Schroeder (42) observed that the outer tangential wall of the epidermal cell is heavily cutinized and that cutin sometimes extends laterally along the radial walls and even continues into the tangential and radial walls of the second and third cell layer below the epidermis. The tangential surface of the epidermis outside the cuticle is overlain with 2 waxy layers. The primary layer is rather uniformly thick, and more or less continuous and homogeneous. The outer layer has a rough and irregular surface and has many fissures and scalings of variable thickness.

The stomata of the avocado fruit are regular in general morphology. Each consists of a pore surrounded by 2 simple guard cells. Schroeder (42) reported that a typical stoma measures 21 microns long by 14 microns wide, with a pore opening approximately 10 microns in length. In young fruits the stomata are normally level with the epidermal layer. In older fruits, however, many of the stomata have been forced upward by lenticel formation beneath them and are no longer visible (10). In smooth-skinned varieties, the lenticels appear on the surface of the fruit as slightly raised, white or gray patches which remain parenchymatous and usually do not break through the epidermis. A slight

amount of cork is formed near the apical end. Rough-skinned varieties, on the other hand, have extensive cork formation in the lenticels, which rupture the epidermis. Several lenticels sometimes coalesce to form large, rough, corky patches (10). The distribution of the lenticels is not uniform, for they are more numerous toward the apex of the fruit. This results from the differential growth of the fruit, which is greatest in the stem end, resulting in wider separation of the lenticels (16).

The fleshy mesocarp of all varieties of avocado fruits exhibits a cellular structure of considerable uniformity. The bulk of the mesocarp is composed of isodiametric parenchyma cells. Schroeder (10) reported that a typical parenchyma cell measures about 60 microns in diameter, has a thin wall, and also contains oil which appears in the form of small droplets. Scattered throughout the parenchyma are specialized oil cells which are distinguished by their large size and lignified, thin cell wall. These cells contain oil in the form of a single large droplet.

Recent electron-microscope studies on the cell-wall structure and intercellular connections in the avocado mesocarp tissue showed that the cellulose microfibrillar pattern of the young cell walls is a reticulum with pores and groups of pores that mark the beginnings of pits. In maturing cells, the walls thicken by the deposition of these microfibrils. The ubiquitous pits in the interparenchymatous cell faces are large, and elliptical or circular in outline. Some pits become partially or completely occluded by microfibrils. The larger ones often remain unobstructed and functional (51).

"The vascular system of the fruit permeates the mesocarp in an asymmetrical pattern, a fact which is linked with the asymmetry of the fruit as a whole" (10). The seed develops slightly off-center, thereby creating a "thick" side and a "thin" side in the fruit. At the region of pedicel attachment, the vascular tissue forms a cylinder. A short distance below, this cylinder is seen to separate into 6 major strands which divide and anastomose until the entire mesocarp is permeated by the vascular network. On the thicker side of the fruit are the well-developed strands that enter the seed coats. Within the seed coats continuous branching, with some anastomosing of the finer bundles again occurs, until a network of conducting tissue surrounds the embryo. On the thinner side are found bundles which lead to the stylar point and some that end blindly toward the apex of the fruit. According to Cummings and Schroeder (10), the xylem of the bundles in the fruit consists principally of strongly lignified annular and helical tracheary elements and of scalariform and simple-pitted vessels. These vascular tissues are sometimes undersirably evident in ripened fruits as stringy, often dark-colored "fibers."

The endocarp, or inner layer of the fruit, is described by the same workers as "a few rows of parenchyma cells which are smaller and somewhat more flattened than the adjoining cells of the mesocarp. These cells lie directly against the outer seed coat and may adhere to it when the seed is removed from the fruit."

The large, conspicuous seed of the avocado is made up of 2 fleshy cotyledons, centrally attached plumule, hypocotyl, and radicle, and 2 papery seed coats closely adherent to each other (10). There is no en-

dosperm left in the seed at maturity. Many irregular cells are reported present in the seed coats. The cotyledons are formed of undifferentiated parenchyma tissue interspersed with occasional idioblasts. Starch is the main storage material of the cotyledons and is present in great abundance.

Developmental Morphology of the Avocado Fruit

Growth of the avocado fruit with special emphasis on the development of the fleshy mesocarp has been studied by Schroeder (45). Several unusual characteristics of morphology and physiology in the avocado fruit were found in this study. He demonstrated that during the early life of the fruit cell division is very rapid and widespread throughout the tissues. A rather marked increase in average cell size is also evident. When the fruit has attained approximately half its ultimate size, most of the individual cells throughout the tissues will have reached their maximum dimensions. Thereafter, the average cell size of the pericarp parenchyma remains practically constant, between 50 and 60 microns, throughout the subsequent life of the fruit. Further increase in fruit size results from continued cell divisions. Mitotic figures and other evidence of current and recent cell division can be observed even in mature avocado fruits.

The property of continued cell division in the avocado fruit has provoked researches which may prove exceptionally useful in future basic studies of fruit morphology and physiology. Such studies have led to discoveries that explants from avocado pericarp can easily be induced to proliferate in vitro (21,48), and the unique demonstration that typical fruit tissue of the avocado can form a successful union by grafting in vivo (49).

The normal gross development of the avocado fruit throughout the season is indicated by a sigmoid curve (29), but more careful and accurate continuous fruit measurements show a series of diurnal fluctuations of contractions during the day and enlargements during the night. Under ordinary conditions the fruit is slightly larger each succeeding morning than on the previous morning (50).

The end of fruit growth is marked by the period of maturation and senescence. The physiological and biochemical processes which underlie the entire life history of the avocado fruit have been studied by several workers, among them Appleman and Noda (2), Biale (7), Burg and Burg (8), and Haas (17).

## MATERIALS AND METHODS

### A. Sample Collection

Six varieties of avocado, ripening at intervals from very early season to late season, were used in this investigation. The varieties were 'Capac' and 'Blake Seedling' belonging to the Mexican race, 'Waldin' and 'Ruehle' of the West Indian race, 'Taylor' of the Guatemalan race, and 'Lula', a Guatemalan x West Indian hybrid. Sample collections were made at approximately 2 week intervals from full bloom until well after commercial maturity.

Full bloom of the very early varieties, 'Capac' and 'Blake Seedling', was recorded as being on January 14 and January 28, 1963, respectively, and "commercial" picking was on June 6 for 'Capac' and July 1 for 'Blake Seedling'. Full bloom for the early, mid-season and late-maturing varieties was estimated to be on March 11, 1963. The early varieties 'Waldin' and 'Ruehle' were commercially mature on August 12. Fruits of the mid-season 'Lula' collected on October 7 ripened satisfactorily. Late-season fruits of the 'Taylor' variety did not satisfy the Florida avocado shipping requirements until October 28 (3).

The materials employed for studies in gross morphology consisted of fruits of the 6 varieties mentioned above. For detailed studies on anatomical changes in the developing fruit only 'Capac', 'Waldin', and 'Taylor' were used. These varieties were selected to insure observable

differences in structure within the fruits. 'Capac', which belongs to the Mexican horticultural race, has a very smooth and paper-thin rind; the 'Waldin' variety of the West Indian horticultural race has a smooth but leathery rind; while 'Taylor', which belongs to the Guatemalan horticultural race, has a rind that is rough and woody.

Samples were collected from mature avocado trees in a state of vigor considered to be desirable for satisfactory fruit production. They were growing at the Sub-tropical Experiment Station of the University of Florida at Homestead, Florida, in Rockdale fine sandy loam soil and had received satisfactory cultural care (23,36). Rainfall for the 1963 growing season was adequate (9).

The fruits were selected at random from all parts of the tree on each sampling date. An effort was always made to select fruits of average size, which were also free from disease and insect infestation. The collected avocado fruits were mailed to Gainesville on the day they were harvested. Soon after arrival, the following gross morphological features were measured: (a) length of long axis from base to apex, (b) diameter of the fruit at its widest part, (c) weight of fruit, (d) fruit volume measured by displacement, (e) pericarp thickness, and (f) length of seed.

The number of fruits measured for each variety at each collection date varied from 10 during the early part of the season to 5 when the fruits were becoming scarce.

### B. Killing and Fixation

Formalin-acetic acid-alcohol (FAA) was used as a killing and

fixing fluid. The standard proportions as outlined by Johansen (20) were used: 90 ml of 50% ethyl alcohol, 5 ml of glacial acetic acid, and 5 ml of formalin.

Flower buds and blossoms were placed in the killing and fixing fluid whole, as were some of the very young fruits until they reached a length of 20 mm. From then until maturity, the fruits were sliced longitudinally through the axis of the fruit from the base to the apex. The fruit slices were preserved in glass jars and stored in the laboratory in FAA solution until used. In the late stages of fruit development, the fruit slices had to be cut into sections of 5 to 10 mm for proper killing, fixation, and storage in the jars. Some of the avocado slices were held in these containers at room temperature for a period of 6 months with no adverse effect upon tissues or cell structure.

This method of fixation made negligible changes in the mean cell diameters. This was ascertained by measuring 50 cylinders of fresh tissue from each of the 3 varieties 'Capac', 'Waldin', and 'Taylor'. The dimensions of these cylinders were again measured 1 month, 3 months, 6 months, and 1 year after fixation in FAA. The largest change in dimensions was only 2.6% and 9 of the 12 determinations changed less than 1%.

### C. Dehydration, Embedding and Sectioning

Selected tissues were divided into smaller pieces for dehydration. The tertiary-butyl alcohol method outlined by Johansen (20) was followed. The dehydrated materials were then imbedded in Fischer Tissuemat and subsequently sectioned with a rotary microtome at from 8 to 18 microns in thickness. These sections were mounted on glass slides that were

smeared with Haupt's adhesive.

Before pieces of the maturing 'Taylor' avocado fruits were subjected to the dehydration process, the woody rinds of the fruit slices had to be softened for a period of from 1 to 2 weeks. Softening was accomplished by soaking the tissues in a mixture of equal parts of glycerol and 50% ethyl alcohol, as was used by Soule (59) for the preparation of mango bud-unions. This procedure successfully softened the woody rinds of 'Taylor' avocado fruits up to the 15th collection, or 28 weeks after full bloom. Perhaps 2-week soakings in glycerol and alcohol were not long enough for the older and tougher rinds, which crumbled and broke out of the paraffin when sectioned. A quick and easy remedy for softening these refractory paraffin-embedded materials was employed. The cutting faces of the paraffin blocks were trimmed to expose the embedded specimens and then soaked in 1% aqueous solution of Sparkleen, a laboratory detergent. Excellent sections were obtained after soaking for 6 to 10 hours. Specimens left in the solution longer than 10 hours were likely to be torn when they were sectioned. Pearlman and Cole (35) successfully softened hard tissue by using other detergents such as Dreft, Glim, and Joy.

Some free-hand sections were cut with a sharp razor blade. These were, of course, thicker by far than those cut with the rotary microtome. However, they did provide valuable comparison with the microtome-sectioned material for getting a true picture of cell size and shape in the developing fruits. The free-hand sections were not included in the photographs.

### D. Staining

Three stains or staining combinations were used: safranin-fast

green FCF, safranin-crystal violet, and Sudan IV. After staining, the sections were mounted in Canada balsam, except those stained with Sudan IV, which were mounted in glycerin jelly.

Safranin-fast green was used for the majority of the slides. This stain was preferred by Johansen (20) and found to be dependable by him for almost every type of plant material. Schroeder (45) used this stain combination in his study of growth and development in the 'Fuerte' avocado fruit. Bell (6) and Mitchell (30) used the same stain combination in their histological studies on the developing fruits of 'Golden Delicious' apple and 'Bartlett' pear, respectively. The safranin-fast green was used in a schedule patterned after that of Bell (6). This stain combination was generally successful, except that in the large mesocarp parenchyma cells of maturing fruits, the cytoplasm stained an intense green color. The cell walls no longer stood out clearly and cell measurements became difficult. It was thought that safranin-crystal violet might give greater contrast in the mesocarp tissue than was accomplished with safranin-fast green, so the schedule recommended by Johansen (20) was followed. This combination frequently overstained the cutinized cells in the exocarp, thus making observations in this tissue system difficult. However, the crystal violet did not stain the cytoplasm of the mesocarp parenchyma very dark, which facilitated cell measurements with the cell walls clearly defined.

Sudan IV has been widely used in previous studies on the outer tissues of fruits. It is a specific fat stain and is considered especially valuable in studies of the cuticle. In this work, Sudan IV was used to define and specifically locate the extent of the cuticle. The schedule of Johansen (20) was used, beginning with material collected

at full bloom. Sudan IV was found to be very successful as a cuticular stain.

## E. Microscopic Study and Cell Measurements

The histological studies were carried out with a compound microscope. This instrument was equipped with a voltage selector to vary light intensity, an adjustable condenser, and a blue light filter.

Cell diameter measurements were made with an eyepiece micrometer disc. Three different magnifications -- 150x, 645x, and 1455x -- were used in this study for the measurement of cells.

Detailed studies of cell size and number in the different tissues of the avocado were made in transverse sections through the middle of the fruit and longitudinal sections through the fruit axis. Comparative cell-diameter measurements and drawings were made from the mid-sections of the fruit, as this has been shown (16) to be the point at which development is most regular.

In selecting representative cells for measurements, those actively dividing, extremely large ones, and exceptionally small cells were avoided. Only those which seemed typical of the different tissues under consideration were measured. From 20 to 25 cells of average diameter were considered to be representative samples of any one tissue on any one collection date. This was in accord with the practice of Bain and Robertson (5), Bell (6), and Schroeder (45) in their studies on the anatomy of developing fruits.

For each cell measured from longitudinally cut fruit tissues, two dimensions were recorded, the longitudinal diameter and the radial

diameter. The transverse-tangential diameters were obtained from transversely cut mid-sections. Cell diameters were measured as closely as possible from middle lamella to middle lamella.

The approximate number of layers of cells in the different tissues of the avocado fruit at various stages of development was ascertained by actually counting the cells of the different tissues mounted on the slides. Those of the thick mesocarp were estimated by dividing the mesocarp by the average radial diameter of the mesocarp cells.

### F. Photomicrography and Illustrations

Representative microscopic slides which best depicted the tissues studied and which were stained sufficiently well were selected for photomicrographing. Comparative outline drawings of the developing fruits were made by tracing whole fruits cut through their longitudinal axis. All drawings of histological details were made at constant magnification with the aid of a camera lucida.

### G. Microchemical Tests

Microchemical tests were carried out to help determine the chemical nature of some anatomical structures. The stone cells with lignified walls in the avocado fruit stained red with the safranin dye used in this study, but so did the suberized walls of the cork cells, the cutinized walls of the epidermal cells, and the tannin contents of some cells. To distinguish the lignified cells from the others and to determine the extent of development of stone-cell clusters, the phloroglucin-HCl test as recommended by Sass (40) was followed. Only lignified walls re-

act with this indicator and become violet-red in color.

Safranin-fast green stained the avocado epidermis and hypodermis red, as well as the filling tissue of lenticels. To determine the chemical nature of the cell walls found in this tissue system, the solubility test of Johansen (20) was employed. Cellulose and lignified cell walls readily dissolved in 50% chromic acid but the suberized walls of cork cells were insoluble.

The cellulose nature of the mesocarp parenchyma cell walls was determined by the iodine-sulphuric acid test of Sass (40). The thick-walled cells in the avocado exocarp were ascertained to be parenchyma when these cells failed to acquire a red color upon immersion in ruthenium red dye as described by Foster (13).

Qualitative determinations of such cell contents as starch, oils, tannins, and crystals were conducted using microchemical reagents recommended by Johansen (20). To distinguish oil globules from vacuoles, the vital dye methylene blue, which penetrates the living cells but accumulates exclusively in the vacuole (14), was employed. The accumulation of this dye in the vacuoles resulted in dark blue coloration of the cells.

## RESULTS

### A. Gross Morphological Development

Volume increase presents a more reliable picture of total growth than does diameter increase. Fig. 1 shows the progressive increase in volume of the 6 different varieties of avocado during the 1963 growing season. The first portions of the curves are similar for all varieties but they differ further along. By superimposing the growth curves it is seen that the fruits of the early-maturing varieties tend to grow continuously, while for those of the later-ripening varieties the growth rate decreases as the season progresses.

If progressive increase in weight is plotted against time, the resulting curve is almost identical to that of volume. The only differences are the slightly more rapid development of weight in the early stages and the slightly slower rates of weight-increase as the fruit approaches maturity. The latter observation is corroborated by changes in specific gravity of the fruit. Young fruits sink in water while the older ones float. The close relationship between volume and weight is true for the almost straight-line growth curve of the early-maturing varieties as well as for the nearly sigmoid curve of the late-season varieties.

Fig. 2 shows graphically the increases in volume, weight, length and width of fruit, and in length of the seed of 'Taylor' avocado throughout the 1963 growing season. Measurements of the longitudinal and radial

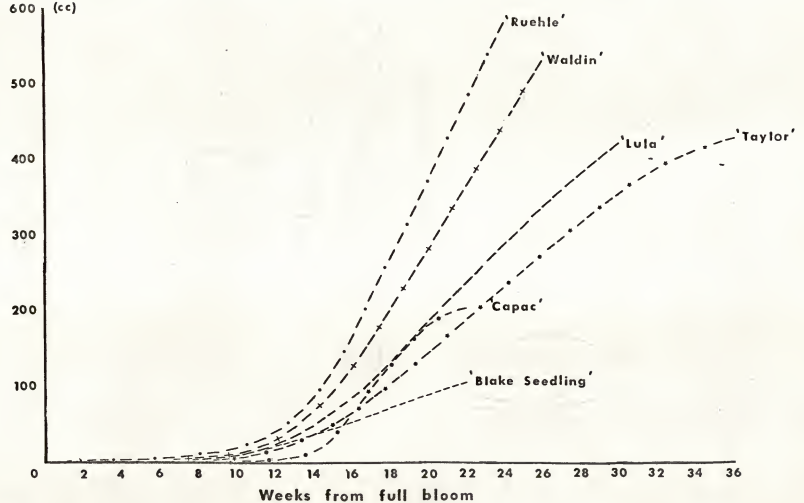



Fig. 1.--Average increase in volume of fruit recorded at bi-weekly intervals from full bloom to fruit maturity.

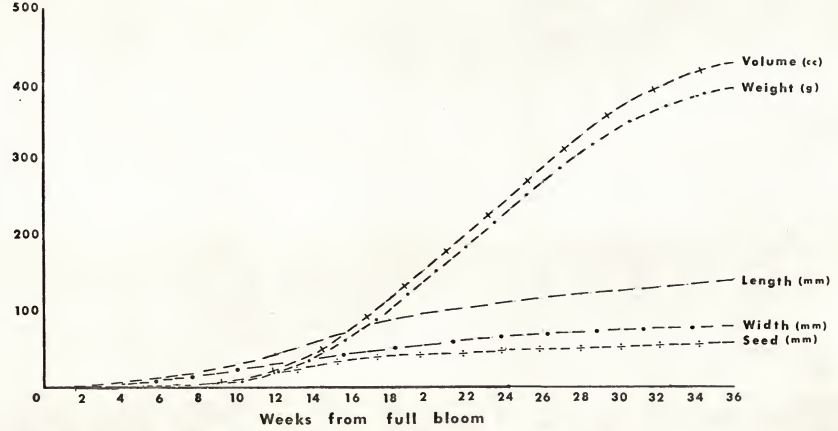



Fig. 2.--Average increase in volume, weight, length, width, and seed of 'Taylor' avocado fruit recorded at bi-weekly intervals from full bloom to fruit maturity.

diameters of the developing fruit give an indication of the morphological changes during the entire growing season. Starting with the abscission of the style, 2 weeks after full bloom, the polar axis maintained a greater dimension than the equatorial diameter. The ratio long axis/short axis was slightly more than 1:1 at the start, but the faster increase in length changed the ratio to almost 2:1 at maturity. The development of the seed is slow and regular.

Tables 1 to 6 show the gross-size development of the 6 varieties of avocado collected during the 1963 growing season from full bloom to maturity. A point of interest is the close similarity of growth patterns of the varieties that mature at the same time. In the late-maturing varieties 'Taylor' and 'Lula', the rate of growth falls off at an early date and the increases in volume, weight, longitudinal diameter, radial diameter, pericarp thickness, and seed length taper off as maturity is approached. The growth features of the 2 West Indian varieties 'Waldin' and 'Ruehle' reflect the same general trend. The relationship between the 2 Mexican varieties 'Capac' and 'Blake Seedling' was not as close as those of the other pairs but was definitely closer to one another than to any variety belonging to a different race.

Fruit shape altered considerably throughout the growing season and its irregularity made estimation of morphological changes difficult. Measurements of fruit characters included in the preceding tables give some indication of these changes, and an exact illustration of changes in shape and size is given in Figures 3 to 47. These show the progressive development of fruits of 'Taylor', 'Waldin' and 'Capac', and also several features not seen in the curves and tables on gross development.

TABLE 1

DEVELOPMENT OF THE 'TAYLOR' AVOCADO FROM FULL BLOOM TO MATURITY

| Pick No. | Date Collected | Weeks From Full Bloom | Volume (cc) | Weight (g) | Length (mm) | Width (mm) | Pericarp Thickness (mm) | Seed Length (mm) |
|---|---|---|---|---|---|---|---|---|
| 1 | 3-11-63 | 0 | 0.001 | 0.0013 | -* | 1.39 | 0.35 | - |
| 2 | 3-25-63 | 2 | 0.001 | 0.0014 | 1.64 | 1.49 | 0.40 | - ** |
| 3 | 4-8-63 | 4 | 0.30 | 0.33 | 10.06 | 7.66 | 1.37 | 2.47 |
| 4 | 4-22-63 | 6 | 2.25 | 2.42 | 20.74 | 13.63 | 3.54 | 7.89 |
| 5 | 5-6-63 | 8 | 4.01 | 4.10 | 27.62 | 16.26 | 4.60 | 8.55 |
| 6 | 5-20-63 | 10 | 6.95 | 7.11 | 33.15 | 20.50 | 5.53 | 11.31 |
| 7 | 6-3-63 | 12 | 16.90 | 17.25 | 45.22 | 26.93 | 7.21 | 16.98 |
| 8 | 6-14-63 | 14 | 53.60 | 53.60 | 71.19 | 40.21 | 9.12 | 30.04 |
| 9 | 7-1-63 | 16 | 97.10 | 93.60 | 86.78 | 47.75 | 11.12 | 35.90 |
| 10 | 7-15-63 | 18 | 110.70 | 107.30 | 89.62 | 50.72 | 11.38 | 38.85 |
| 11 | 7-29-63 | 20 | 150.00 | 144.00 | 98.06 | 56.70 | 12.98 | 42.60 |
| 12 | 8-12-63 | 22 | 197.00 | 191.90 | 103.28 | 63.10 | 13.18 | 45.60 |
| 13 | 8-26-63 | 24 | 275.60 | 266.80 | 110.02 | 71.36 | 13.94 | 50.78 |
| 14 | 9-9-63 | 26 | 276.80 | 271.40 | 118.54 | 72.40 | 14.50 | 51.38 |
| 15 | 9-23-63 | 28 | 312.00 | 306.00 | 121.62 | 73.84 | 15.24 | 54.54 |
| 16 | 10-7-63 | 30 | 388.40 | 379.18 | 128.04 | 81.02 | 15.64 | 57.40 |
| 17 | 10-21-63 | 32 | 413.20 | 379.22 | 138.62 | 78.20 | 15.98 | 59.28 |
| 18 | 11-4-63 | 34 | 388.20 | 371.40 | 126.82 | 81.14 | 16.24 | 55.14 |
| 19 | 11-18-63 | 36 | 428.40 | 396.74 | 143.50 | 81.70 | 16.64 | 53.48 |

*Style has not yet abscissed.

**The seed could not be identified until this date without the aid of a microscope.

TABLE 2

DEVELOPMENT OF THE 'LULA' AVOCADO FROM FULL BLOOM TO MATURITY

| Pick No. | Date Collected | Weeks From Full Bloom | Volume (cc) | Weight (g) | Length (mm) | Width (mm) | Pericarp Thickness (mm) | Seed Length (mm) |
|---|---|---|---|---|---|---|---|---|
| 1 | 3-11-63 | 0 | 0.001 | 0.0012 | -* | 1.34 | 0.35 | - |
| 2 | 3-25-63 | 2 | 0.001 | 0.0015 | 1.63 | 1.42 | 0.50 | - |
| 3 | 4-8-63 | 4 | 0.18 | 0.20 | 7.82 | 5.92 | 1.55 | 2.57** |
| 4 | 4-22-63 | 6 | 2.00 | 2.11 | 20.11 | 13.13 | 3.51 | 7.04 |
| 5 | 5-6-63 | 8 | 6.05 | 6.16 | 31.48 | 19.09 | 5.41 | 11.78 |
| 6 | 5-20-63 | 10 | 21.50 | 21.54 | 48.68 | 29.18 | 7.83 | 19.08 |
| 7 | 6-3-63 | 12 | 37.20 | 38.41 | 63.98 | 35.82 | 8.38 | 25.53 |
| 8 | 6-14-63 | 14 | 69.10 | 70.91 | 79.68 | 44.79 | 9.75 | 32.15 |
| 9 | 7-1-63 | 16 | 81.20 | 78.60 | 82.51 | 45.83 | 10.22 | 37.96 |
| 10 | 7-15-63 | 18 | 138.60 | 135.90 | 101.51 | 55.74 | 11.89 | 41.35 |
| 11 | 7-29-63 | 20 | 191.00 | 184.40 | 108.66 | 62.34 | 12.74 | 45.42 |
| 12 | 8-12-63 | 22 | 233.00 | 226.40 | 113.60 | 67.74 | 13.88 | 46.32 |
| 13 | 8-26-63 | 24 | 292.00 | 255.00 | 117.58 | 74.40 | 14.80 | 51.14 |
| 14 | 9-9-63 | 26 | 338.00 | 333.80 | 124.28 | 78.52 | 15.10 | 53.72 |
| 15 | 9-23-63 | 28 | 344.20 | 339.20 | 130.02 | 78.66 | 14.82 | 56.52 |
| 16 | 10-7-63 | 30 | 421.40 | 411.48 | 134.52 | 84.24 | 14.86 | 60.00 |

*Style has not yet abscissed.

**The seed could not be identified until this date without the aid of a microscope.

TABLE 3

DEVELOPMENT OF THE 'WALDIN' AVOCADO FROM FULL BLOOM TO MATURITY

| Pick No. | Date Collected | Weeks from Full Bloom | Volume (cc) | Weight (g) | Length (mm) | Width (mm) | Pericarp Thickness (mm) | Seed Length (mm) |
|---|---|---|---|---|---|---|---|---|
| 1 | 3-11-63 | 0 | 0.001 | 0.0010 | -* | 1.18 | 0.28 | - |
| 2 | 3-25-63 | 2 | 0.001 | 0.0013 | 1.30 | 1.39 | 0.37 | - |
| 3 | 4-8-63 | 4 | 0.30 | 0.33 | 9.68 | 7.32 | 1.33 | 3.95** |
| 4 | 4-22-63 | 6 | 2.05 | 2.06 | 18.77 | 13.14 | 3.43 | 8.86 |
| 5 | 5-6-63 | 8 | 9.05 | 9.17 | 33.12 | 21.98 | 5.74 | 14.84 |
| 6 | 5-20-63 | 10 | 15.30 | 15.41 | 38.08 | 25.88 | 6.84 | 16.33 |
| 7 | 6-3-63 | 12 | 30.05 | 30.49 | 49.52 | 33.38 | 7.31 | 24.18 |
| 8 | 6-14-63 | 14 | 84.50 | 85.23 | 69.91 | 48.14 | 8.82 | 32.97 |
| 9 | 7-1-63 | 16 | 122.40 | 118.30 | 73.53 | 55.87 | 12.71 | 33.15 |
| 10 | 7-15-63 | 18 | 242.60 | 238.60 | 104.34 | 68.92 | 14.20 | 45.60 |
| 11 | 7-29-63 | 20 | 294.80 | 289.30 | 102.00 | 77.00 | 14.46 | 47.86 |
| 12 | 8-12-63 | 22 | 354.60 | 347.10 | 111.46 | 80.92 | 16.40 | 49.08 |
| 13 | 8-26-63 | 24 | 456.00 | 449.00 | 112.50 | 90.34 | 17.70 | 52.60 |
| 14*** | 9-9-63 | 26 | 531.40 | 516.60 | 121.40 | 94.52 | 17.84 | 56.00 |

*Style has not yet abscissed.

**The seed could not be identified until this date without the aid of a microscope.

***Extra collection, four weeks after first maturity.

TABLE 4

DEVELOPMENT OF THE 'RUEHLE' AVOCADO FROM FULL BLOOM TO MATURITY

| Pick No. | Date Collected | Weeks from Full Bloom | Volume (cc) | Weight (g) | Length (mm) | Width (mm) | Pericarp Thickness (mm) | Seed Length (mm) |
|---|---|---|---|---|---|---|---|---|
| 1 | 3-11-63 | 0 | 0.001 | 0.0013 | -* | 1.36 | 0.34 | - |
| 2 | 3-25-63 | 2 | 0.05 | 0.06 | 4.83 | 3.85 | 1.40 | - |
| 3 | 4-8-63 | 4 | 0.88 | 0.95 | 13.75 | 10.15 | 2.55 | 5.79** |
| 4 | 4-22-63 | 6 | 7.10 | 7.24 | 30.16 | 19.79 | 4.99 | 12.55 |
| 5 | 5-6-63 | 8 | 13.95 | 14.09 | 37.21 | 25.19 | 6.38 | 14.22 |
| 6 | 5-20-63 | 10 | 16.90 | 16.94 | 38.84 | 27.16 | 6.48 | 17.00 |
| 7 | 6-3-63 | 12 | 42.80 | 42.36 | 52.76 | 33.53 | 8.05 | 22.31 |
| 8 | 6-14-63 | 14 | 60.19 | 59.40 | 74.20 | 42.58 | 8.84 | 27.61 |
| 9 | 7-1-63 | 16 | 159.30 | 156.40 | 89.03 | 60.40 | 12.66 | 34.54 |
| 10 | 7-15-63 | 18 | 315.60 | 306.80 | 111.92 | 77.00 | 14.40 | 43.26 |
| 11 | 7-29-63 | 20 | 361.00 | 351.10 | 118.98 | 81.18 | 18.14 | 44.60 |
| 12 | 8-12-63 | 22 | 444.60 | 428.48 | 123.60 | 85.98 | 19.34 | 49.26 |
| 13 | 8-26-63 | 24 | 579.80 | 543.06 | 137.76 | 95.46 | 21.16 | 48.74 |

*Style has not yet abscissed.

**The seed could not be identified until this date without the aid of a microscope.

TABLE 5

DEVELOPMENT OF THE 'CAPAC' AVOCADO FROM FULL BLOOM TO MATURITY

| Pick No. | Date Collected | Weeks from Full Bloom | Volume (cc) | Weight (g) | Length (mm) | Width (mm) | Pericarp Thickness (mm) | Seed Length (mm) |
|---|---|---|---|---|---|---|---|---|
| 1 | 1-14-63 | 0 | 0.001 | 0.0011 | -* | 1.21 | 0.34 | - |
| 2 | 1-28-63 | 2 | 0.010 | 0.012 | 4.07 | 2.70 | 0.43 | - |
| 3 | 2-11-63 | 4 | 0.030 | 0.033 | 4.70 | 3.68 | 1.32 | 2.00* |
| 4 | 2-26-63 | 6 | 0.23 | 0.25 | 9.68 | 7.21 | 2.00 | 2.83 |
| 5 | 3-11-63 | 8 | 1.08 | 1.13 | 13.28 | 9.72 | 2.30 | 6.32 |
| 6 | 3-25-63 | 10 | 1.75 | 1.81 | 15.38 | 10.69 | 3.10 | 7.40 |
| 7 | 4-8-63 | 12 | 2.35 | 2.50 | 20.45 | 13.42 | 3.58 | 9.06 |
| 8 | 4-22-63 | 14 | 6.80 | 6.82 | 31.35 | 19.10 | 5.80 | 13.30 |
| 9 | 5-6-63 | 16 | 75.00 | 74.22 | 68.48 | 46.25 | 9.80 | 33.24 |
| 10 | 5-20-63 | 18 | 108.00 | 106.88 | 77.76 | 53.36 | 10.82 | 36.98 |
| 11 | 6-3-63 | 20 | 205.00 | 198.88 | 93.05 | 66.72 | 12.90 | 42.92 |
| 12 | 6-14-63 | 22 | 204.00 | 202.06 | 95.38 | 65.76 | 13.28 | 46.22 |

*Style has not yet abscissed.

**The seed could not be identified until this date without the aid of a microscope.

TABLE 6

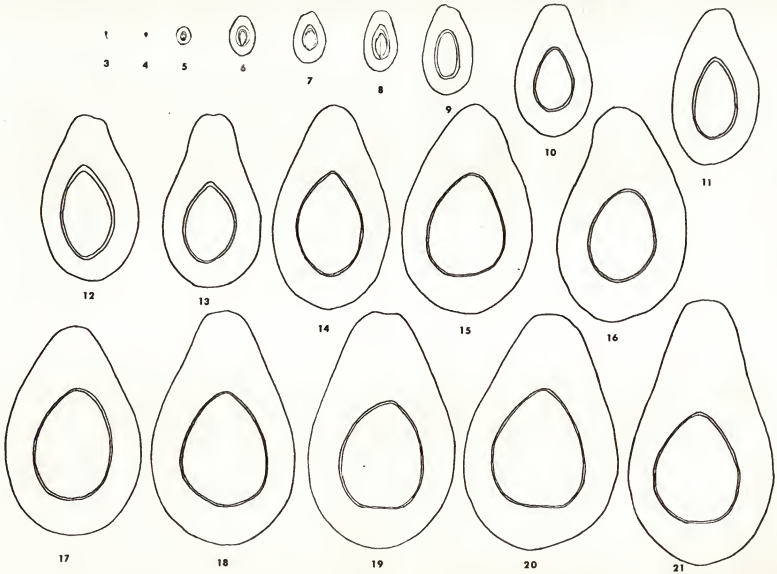
DEVELOPMENT OF THE 'BLAKE SEEDLING' AVOCADO FROM FULL BLOOM TO MATURITY

| Pick No. | Date Collected | Weeks from Full Bloom | Volume (cc) | Weight (g) | Length (mm) | Width (mm) | Pericarp Thickness (mm) | Seed Length (mm) |
|---|---|---|---|---|---|---|---|---|
| 1 | 1-28-63 | 0 | 0.001 | 0.002 | -* | 1.64 | 0.41 | - |
| 2 | 2-11-63 | 2 | 0.015 | 0.02 | 4.01 | 3.16 | 0.60 | - |
| 3 | 2-25-63 | 4 | 0.13 | 0.14 | 8.18 | 6.03 | 1.82 | 2.83** |
| 4 | 3-11-63 | 6 | 1.11 | 1.18 | 15.55 | 10.57 | 2.45 | 5.48 |
| 5 | 3-25-63 | 8 | 5.49 | 5.61 | 24.84 | 16.05 | 3.30 | 13.83 |
| 6 | 4-8-63 | 10 | 12.60 | 12.94 | 44.02 | 23.59 | 5.27 | 17.97 |
| 7 | 4-22-63 | 12 | 23.80 | 24.13 | 53.55 | 29.79 | 6.43 | 22.13 |
| 8 | 5-6-63 | 14 | 36.20 | 36.54 | 64.33 | 34.36 | 6.98 | 27.80 |
| 9 | 5-20-63 | 16 | 44.00 | 44.70 | 71.80 | 37.10 | 7.92 | 27.88 |
| 10 | 6-3-63 | 18 | 74.80 | 72.58 | 82.80 | 43.50 | 8.90 | 35.12 |
| 11 | 6-14-63 | 20 | 81.60 | 83.05 | 85.70 | 46.07 | 8.96 | 36.18 |
| 12 | 7-1-63 | 22 | 107.20 | 104.60 | 91.13 | 49.61 | 10.15 | 39.13 |

*Style has not yet abscissed.

**The seed could not be identified until this date without the aid of a microscope.

Figs. 3-21.--Tracings of 'Taylor' avocado in longisection from full bloom to fruit maturity: Fig. 3, full bloom; Fig. 4, 2 weeks after full bloom; Fig. 5, 4 weeks; Fig. 6, 6 weeks; Fig. 7, 8 weeks; Fig. 8, 10 weeks; Fig. 9, 12 weeks; Fig. 10, 14 weeks; Fig. 11, 16 weeks; Fig. 12, 18 weeks; Fig. 13, 20 weeks; Fig. 14, 22 weeks; Fig. 15, 24 weeks; Fig. 16, 26 weeks; Fig. 17, 28 weeks; Fig. 18, 30 weeks; Fig. 19, 32 weeks; Fig. 20, 34 weeks; Fig. 21, 36 weeks. All drawings are ½ actual size.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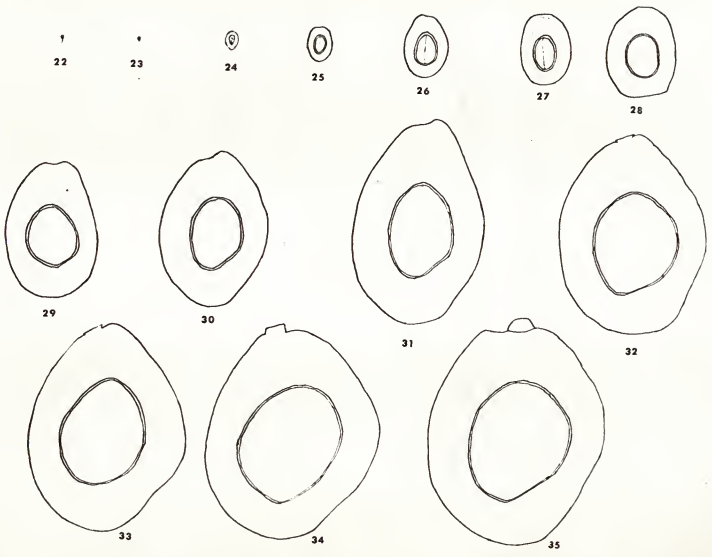
Figs. 22-35.--Tracings of 'Waldin' avocado in longisection from full bloom to fruit maturity: Fig. 22, full bloom; Fig. 23, 2 weeks after full bloom; Fig. 24, 4 weeks; Fig. 25, 6 weeks; Fig. 26, 8 weeks; Fig. 27, 10 weeks; Fig. 28, 12 weeks; Fig. 29, 14 weeks; Fig. 30, 16 weeks; Fig. 31, 18 weeks; Fig. 32, 20 weeks; Fig. 33, 22 weeks; Fig. 34, 24 weeks; Fig. 35, 26 weeks. All drawings are ½ actual size.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Figs. 36-47.--Tracings of 'Capac' avocado in longisection from full bloom to fruit maturity: Fig. 36, full bloom; Fig. 37, 2 weeks after full bloom; Fig. 38, 4 weeks; Fig. 39, 6 weeks; Fig. 40, 8 weeks; Fig. 41, 10 weeks; Fig. 42, 12 weeks; Fig. 43, 14 weeks; Fig. 44, 16 weeks; Fig. 45, 18 weeks; Fig. 46, 20 weeks; Fig. 47, 22 weeks. All drawings are ½ actual size.

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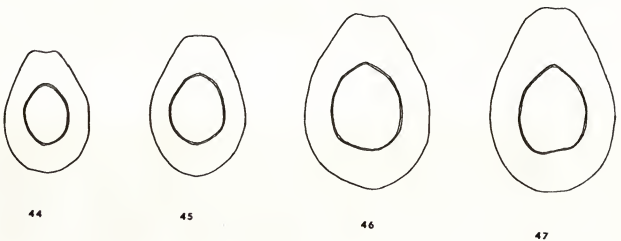
44
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47

Fruit-size increase occurs in all parts simultaneously and continuously through the growing season. This observation concurs with that of Cummings and Schroeder (10) in their study on the 'Fuerte' avocado in California. The fruits develop slightly asymmetrically. The side where the well-developed vascular bundles enter the seed coats is thicker than the opposite side where the vascular bundles end blindly in the fruit apex.

The seed could not be identified without the aid of a microscope until after the fourth week following full bloom. At this stage of fruit development, the endosperm has already filled up the cavity while the young embryo is still emerging from the chalazal end of the embryo sac. As the young fruits grow older, the embryo continues to grow while the endosperm degenerates. The embryo with its large and fleshy cotyledons eventually fills up the cavity. Endosperm was no longer found in fruits 3 months or more old.

## B. Histological Development

Histological observations were made on the varieties 'Taylor', 'Waldin', and 'Capac', each representing one of the 3 recognized races of avocado. Cell-diameter measurements were made in all tissues of the fruit, beginning with the epidermis and working inward to the endocarp. Size and number of cells in the different tissue systems are given in Tables 7 to 12. Histological details at monthly intervals from full bloom to maturity are shown in Figures 48 to 119.

With safranin-fast green stain, the cuticle of avocado fruits usually took a light pink color. Tannin in the epidermal cells, de-

TABLE 7

CHANGES IN CELL SIZE (MICRONS) OF THE DIFFERENT TISSUES OF DEVELOPING 'TAYLOR' AVOCADO FRUITS

| Weeks from full bloom | Cuticle Thickness | Epidermis | | | Hypodermis | | | Mesocarp Parenchyma | | | Endocarp | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Longitudinal diameter | Radial diameter | Transverse-tangential diameter | Longitudinal diameter | Radial diameter | Transverse-tangential diameter | Longitudinal diameter | Radial diameter | Transverse-tangential diameter | Longitudinal diameter | Radial diameter | Transverse-tangential diameter |
| 0 | (trace) | 14 | 16 | 15 | 18 | 13 | 20 | 26 | 22 | 24 | 9 | 7 | 8 |
| 2 | 1.0 | 14 | 18 | 14 | 21 | 15 | 22 | 28 | 23 | 26 | 9 | 8 | 8 |
| 4 | 2.0 | 12 | 23 | 13 | 22 | 16 | 22 | 30 | 28 | 28 | 9 | 10 | 8 |
| 6 | 3.0 | 11 | 24 | 13 | 23 | 17 | 23 | 35 | 35 | 35 | 10 | 12 | 9 |
| 8 | 4.0 | 11 | 26 | 13 | 25 | 17 | 25 | 38 | 38 | 38 | 10 | 13 | 10 |
| 10 | 5.0 | 12 | 28 | 13 | 27 | 18 | 27 | 42 | 41 | 42 | 11 | 13 | 10 |
| 12 | 5.5 | 13 | 30 | 13 | 29 | 19 | 28 | 44 | 44 | 44 | 12 | 13 | 11 |
| 14 | 6.0 | 14 | 30 | 13 | 31 | 19 | 30 | 47 | 45 | 46 | 13 | 13 | 13 |
| 16 | 6.0 | 14 | 33 | 13 | 31 | 19 | 32 | 50 | 51 | 51 | 15 | 13 | 15 |
| 18 | 6.0 | 14 | 34 | 14 | 32 | 19 | 33 | 55 | 53 | 55 | 17 | 15 | 20 |
| 20 | 6.5 | 16 | 34 | 16 | 35 | 19 | 36 | 59 | 57 | 60 | 22 | 24 | 23 |
| 22 | 7.0 | 16 | 35 | 16 | 35 | 19 | 36 | 62 | 59 | 63 | 24 | 28 | 25 |
| 24 | 7.0 | 16 | 37 | 16 | 36 | 20 | 37 | 65 | 63 | 65 | 27 | 32 | 27 |
| 26 | 8.0 | 16 | 38 | 17 | 38 | 21 | 38 | 66 | 63 | 66 | 31 | 34 | 32 |
| 28 | 8.0 | 17 | 38 | 17 | 38 | 22 | 38 | 67 | 66 | 66 | 32 | 39 | 33 |
| 30 | 8.0 | 17 | 38 | 17 | 39 | 23 | 39 | 68 | 66 | 67 | 34 | 41 | 34 |
| 32 | 8.0 | 17 | 39 | 17 | 39 | 23 | 39 | 69 | 66 | 68 | 36 | 42 | 36 |
| 34 | 8.0 | 17 | 39 | 17 | 41 | 23 | 40 | 70 | 66 | 69 | 36 | 44 | 37 |
| 36 (mature) | 8.0 | 17 | 40 | 17 | 41 | 23 | 41 | 72 | 67 | 69 | 36 | 44 | 37 |
| 36 (ripe) | 8.0 | 17 | 40 | 17 | 41 | 23 | 41 | 67 | 64 | 68 | 36 | 44 | 37 |

TABLE 8

CHANGES IN CELL SIZE (MICRONS) OF THE DIFFERENT TISSUES OF DEVELOPING 'WALDIN' AVOCADO FRUITS

| Weeks from full bloom | Cuticle Thickness | Epidermis | | | Hypodermis | | | Mesocarp Parenchyma | | | Endocarp | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Longitudinal diameter | Radial diameter | Transverse-tangential diameter | Longitudinal diameter | Radial diameter | Transverse-tangential diameter | Longitudinal diameter | Radial diameter | Transverse-tangential diameter | Longitudinal diameter | Radial diameter | Transverse-tangential diameter |
| 0 | (trace) | 14 | 14 | 10 | 19 | 14 | 14 | 26 | 20 | 18 | 7 | 8 | 6 |
| 2 | 1.5 | 14 | 16 | 10 | 19 | 18 | 17 | 25 | 21 | 21 | 7 | 8 | 6 |
| 4 | 2.0 | 12 | 21 | 10 | 18 | 18 | 16 | 26 | 23 | 28 | 7 | 9 | 6 |
| 6 | 3.0 | 10 | 20 | 10 | 18 | 18 | 20 | 32 | 27 | 29 | 7 | 12 | 8 |
| 8 | 3.5 | 11 | 25 | 11 | 19 | 19 | 22 | 38 | 35 | 37 | 11 | 12 | 12 |
| 10 | 5.0 | 11 | 25 | 12 | 22 | 19 | 22 | 42 | 39 | 39 | 15 | 12 | 13 |
| 12 | 5.0 | 12 | 27 | 12 | 24 | 19 | 23 | 46 | 42 | 48 | 15 | 17 | 15 |
| 14 | 5.0 | 12 | 31 | 12 | 29 | 20 | 24 | 46 | 44 | 50 | 17 | 17 | 15 |
| 16 | 5.5 | 14 | 34 | 14 | 30 | 20 | 27 | 51 | 47 | 56 | 17 | 17 | 17 |
| 18 | 6.0 | 17 | 34 | 17 | 30 | 20 | 29 | 61 | 55 | 60 | 17 | 18 | 18 |
| 20 | 6.0 | 18 | 35 | 17 | 34 | 21 | 32 | 63 | 59 | 61 | 18 | 19 | 19 |
| 22 | 6.0 | 18 | 35 | 17 | 37 | 21 | 33 | 65 | 60 | 64 | 20 | 19 | 21 |
| 24 | 7.0 | 20 | 36 | 18 | 37 | 21 | 34 | 67 | 60 | 66 | 22 | 20 | 21 |
| 26 (mature) | 7.0 | 23 | 37 | 21 | 39 | 22 | 37 | 68 | 61 | 67 | 23 | 21 | 21 |
| 26 (ripe) | 7.0 | 22 | 37 | 21 | 38 | 22 | 38 | 61 | 56 | 65 | 23 | 21 | 21 |

TABLE 9

CHANGES IN CELL SIZE (MICRONS) OF THE DIFFERENT TISSUES OF DEVELOPING 'CAPAC' AVOCADO FRUITS

| | | Epidermis | | | Hypodermis | | | Mesocarp Parenchyma | | | Endocarp | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Weeks from full bloom | Cuticle Thickness | Longitudinal diameter | Radial diameter | Transverse-tangential diameter | Longitudinal diameter | Radial diameter | Transverse-tangential diameter | Longitudinal diameter | Radial diameter | Transverse-tangential diameter | Longitudinal diameter | Radial diameter | Transverse-tangential diameter |
| 0 | (trace) | 15 | 18 | 15 | 21 | 14 | 18 | 24 | 22 | 22 | 9 | 8 | 9 |
| 2 | 1.5 | 13 | 20 | 12 | 21 | 15 | 20 | 26 | 24 | 26 | 9 | 8 | 9 |
| 4 | 2.0 | 12 | 21 | 12 | 21 | 15 | 21 | 28 | 28 | 26 | 10 | 10 | 9 |
| 6 | 3.0 | 12 | 22 | 13 | 23 | 15 | 21 | 31 | 28 | 29 | 10 | 11 | 9 |
| 8 | 4.0 | 12 | 22 | 13 | 26 | 15 | 24 | 35 | 30 | 34 | 12 | 11 | 11 |
| 10 | 5.0 | 12 | 25 | 13 | 26 | 15 | 24 | 36 | 34 | 35 | 12 | 11 | 111 |
| 12 | 5.0 | 13 | 25 | 13 | 26 | 15 | 24 | 39 | 37 | 37 | 12 | 12 | 11 |
| 14 | 5.0 | 13 | 25 | 13 | 26 | 16 | 27 | 41 | 41 | 42 | 13 | 12 | 12 |
| 16 | 6.0 | 14 | 26 | 13 | 26 | 16 | 28 | 55 | 53 | 55 | 16 | 18 | 25 |
| 18 | 6.0 | 16 | 26 | 16 | 32 | 16 | 33 | 67 | 63 | 65 | 23 | 27 | 22 |
| 20 | 7.0 | 16 | 26 | 16 | 37 | 17 | 35 | 70 | 65 | 66 | 26 | 31 | 29 |
| 22 | 7.0 | 17 | 26 | 18 | 38 | 18 | 36 | 70 | 63 | 68 | 26 | 33 | 31 |

TABLE 10

APPROXIMATE NUMBER OF LAYERS OF CELLS IN DIFFERENT TISSUES OF THE DEVELOPING 'TAYLOR' AVOCADO FRUIT

| Weeks from full bloom | Epidermis | Hypodermis | Mesocarp | Endocarp |
|---|---|---|---|---|
| 0 | 1 | 1-2 | 14 | 1 |
| 2 | 1 | 1-2 | 16 | 1 |
| 4 | 1 | 2-3 | 45 | 1 |
| 6 | 1 | 2-3 | 87 | 1 |
| 8 | 1 | 2-3 | 108 | 1 |
| 10 | 1 | 2-3 | 121 | 1 |
| 12 | 1 | 2-4 | 149 | 1 |
| 14 | 1 | 2-4 | 155 | 1 |
| 16 | 1 | 2-5 | 179 | 1 |
| 18 | 1-2 | 2-6 | 190 | 1 |
| 20 | 1-2 | 2-6 | 202 | 1 |
| 22 | 1-2 | 2-6 | 203 | 1 |
| 24 | 1-2 | 2-6 | 205 | 1 |
| 26 | 1-2 | 2-6 | 211 | 1 |
| 28 | 1-2 | 2-6 | 214 | 1 |
| 30 | 1-2 | 2-6 | 217 | 1 |
| 32 | 1-2 | 2-6 | 221 | 1 |
| 34 | 1-2 | 2-6 | 223 | 1 |
| 36 | 1-2 | 2-6 | 231 | 1 |

TABLE 11

APPROXIMATE NUMBER OF LAYERS OF CELLS IN DIFFERENT TISSUES OF THE DEVELOPING 'WALDIN' AVOCADO FRUIT

| Weeks from full bloom | Epidermis | Hypodermis | Mesocarp | Endocarp |
|---|---|---|---|---|
| 0 | 1 | 1-2 | 12 | 1 |
| 2 | 1 | 1-2 | 14 | 1 |
| 4 | 1 | 2-3 | 54 | 1 |
| 6 | 1 | 2-3 | 104 | 1 |
| 8 | 1 | 2-4 | 146 | 1 |
| 10 | 1 | 2-5 | 154 | 1 |
| 12 | 1 | 2-5 | 162 | 1 |
| 14 | 1 | 2-5 | 180 | 1 |
| 16 | 1-2 | 2-5 | 192 | 1 |
| 18 | 1-2 | 2-5 | 205 | 1 |
| 20 | 1-2 | 2-5 | 216 | 1 |
| 22 | 1-2 | 2-5 | 232 | 1 |
| 24 | 1-2 | 2-5 | 246 | 1 |
| 26 | 1-2 | 2-5 | 268 | 1 |

TABLE 12

APPROXIMATE NUMBER OF LAYERS OF CELLS IN DIFFERENT TISSUES OF THE DEVELOPING 'CAPAC' AVOCADO FRUIT

| Weeks from full bloom | Epidermis | Hypodermis | Mesocarp | Endocarp |
|---|---|---|---|---|
| 0 | 1 | 1-2 | 15 | 1 |
| 2 | 1 | 1-2 | 16 | 1 |
| 4 | 1 | 2-3 | 34 | 1 |
| 6 | 1 | 2-3 | 65 | 1 |
| 8 | 1 | 2-3 | 70 | 1 |
| 10 | 1 | 2-3 | 84 | 1 |
| 12 | 1 | 2-3 | 80 | 1 |
| 14 | 1 | 2-4 | 116 | 1 |
| 16 | 1-2 | 2-4 | 137 | 1 |
| 18 | 1-2 | 2-4 | 165 | 1 |
| 20 | 1-2 | 2-5 | 192 | 1 |
| 22 | 1-2 | 2-5 | 200 | 1 |

veloping hypodermis, exocarp parenchyma, and a few mesocarp cells close to the vascular bundles stained deep red. The xylem elements, the stone cells in the exocarp, and the endocarp at maturity stained red, while the cellulose walls and cytoplasm of the remaining tissues of the fruit stained green in varying degrees. With safranin-crystal violet, only lignified cells stained red.

Exocarp

The exocarp is the layer which is removed when the fruit is peeled. The avocado exocarp is a composite structure consisting of epidermal, parenchyma, and sclerenchyma tissues. A thin cuticle forms a wax-like film over the surface of the fruit (10). In the very young fruit, the ovary is covered with a mat of hairs. The epidermal hairs in the avocado are simple and unicellular, and are about 120 to 150 microns long.

Epidermal Hairs.--At full bloom, the pistils of 'Taylor' are entirely covered by a dense mat of epidermal hairs (Figs. 48,49). Each hair is living, with a well-defined nucleus located towards the middle portion. Epidermal hairs also cover the entire pistil of 'Waldin' at full bloom but they are not as dense. More of them are located towards the stylar end than towards the base. Epidermal hairs on 'Capac' pistils are few and very sparsely located.

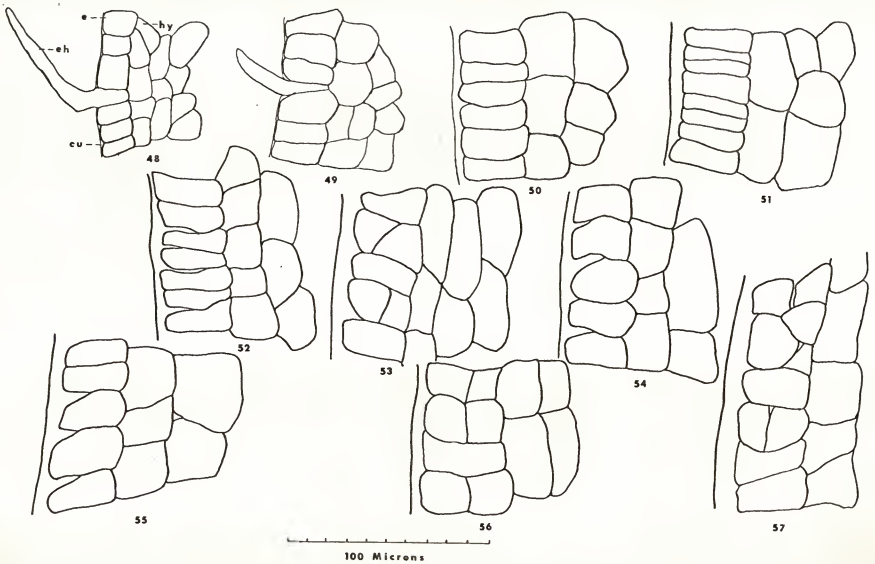
Two weeks after full bloom, the hairs in 'Taylor' become slightly separated as a result of the multiplication of the regular epidermal cells. The cell contents are inconspicuous and as living cells, the hairs are now becoming inactive. Hairs are no longer found at the basal portion of the young 'Waldin' fruits, although a few can still be observed towards the apical end. In 'Capac', the epidermal hairs are gone.

One month after full bloom, the cuticle can be observed spreading over the side walls of the small bases of the hairs within the epidermal layer. The distal portions of these hairs are starting to slough off. At this stage,there are a few intact hairs left in 'Taylor' and very few in 'Waldin'. In the latter,the hair bases are filled with cutin and the epidermal hairs are apparently dead.

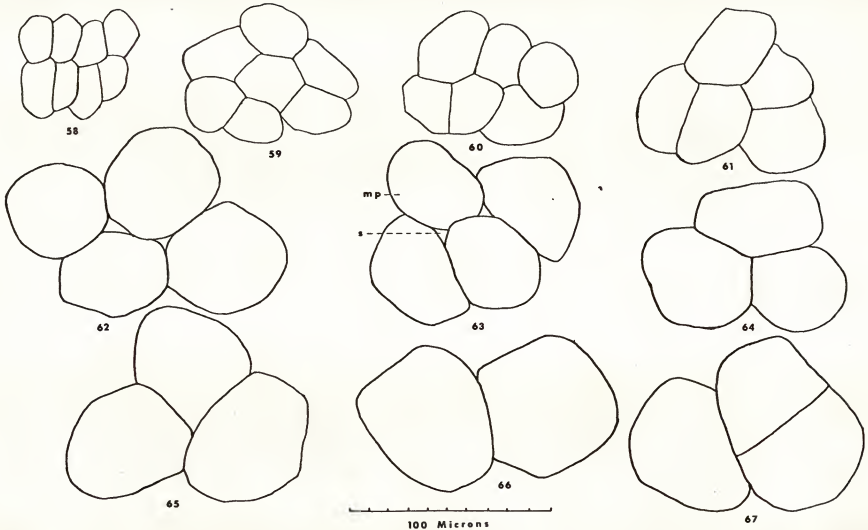
On 6-weeks-old 'Taylor' fruits, no more intact hairs can be observed. The only evidence of their presence in the earlier stages of fruit development is the numerous stubs, bases of which are filled in with cutin. The epidermal hairs are entirely gone in the 'Waldin' variety at this age.

Two months after full bloom, the epidermal hairs of 'Taylor' are all gone also.

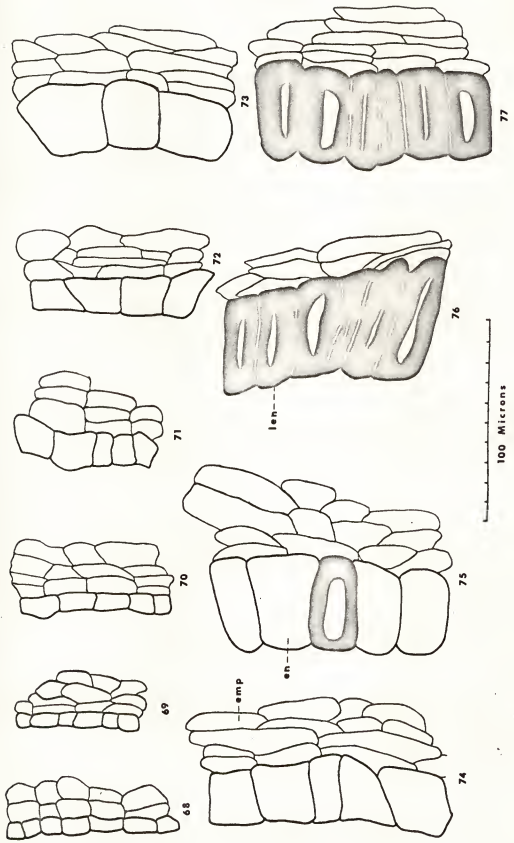
Figs. 48-57.--Cellular changes in epidermis and hypodermis of 'Taylor' avocado from full bloom to fruit maturity: Fig. 48, full bloom; Fig. 49, 1 month after full bloom; Fig. 50, 2 months; Fig. 51, 3 months; Fig. 52, 4 months; Fig. 53, 5 months; Fig. 54, 6 months; Fig. 55, 7 months; Fig. 56, 8 months; Fig. 57, 9 months. eh, epidermal hair; cu, cuticle; e, epidermis; hy, hypodermis.


e
hy
eh
cu
48
49
50
51
52
53
54
55
56
57
100 Microns

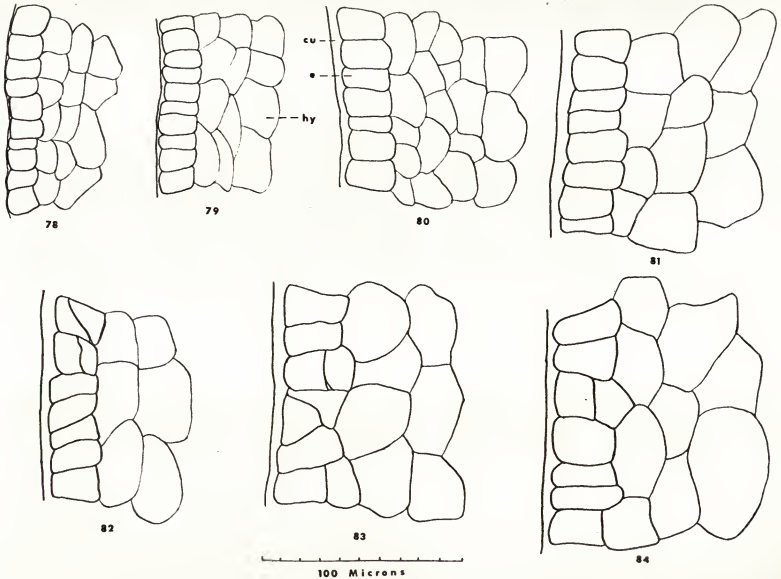
Figs. 58-67.--Cellular changes in the mesocarp of 'Taylor' avocado from full bloom to fruit maturity: Fig. 58, full bloom; Fig. 59, 1 month after full bloom; Fig. 60, 2 months; Fig. 61, 3 months; Fig. 62, 4 months; Fig. 63, 5 months; Fig. 64, 6 months; Fig. 65, 7 months; Fig. 66, 8 months; Fig. 67, 9 months. mp, mesocarp parenchyma; s, intercellular space.


58
59
60
61
mp
s
62
63
64
65
66
67
100 Microns

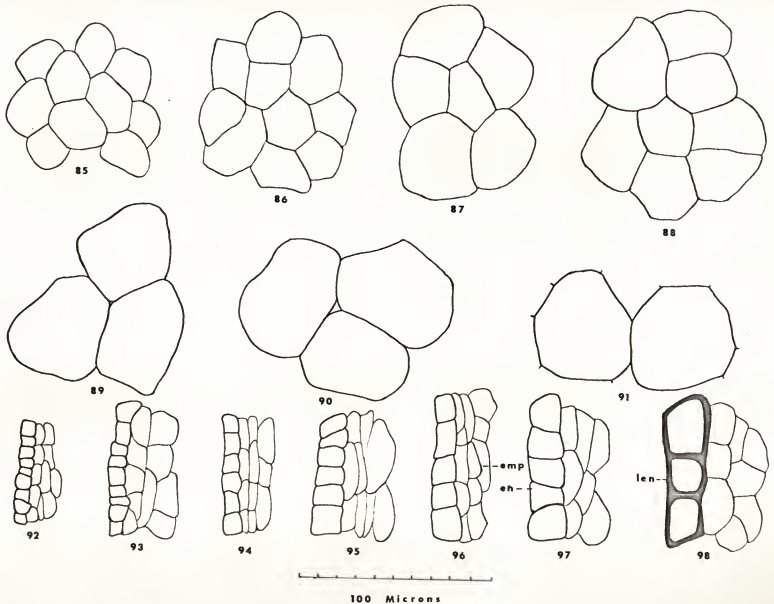
Figs. 68-77.--Cellular changes in the endocarp of 'Taylor' avocado from full bloom to fruit maturity: Fig. 68, full bloom; Fig. 69, 1 month after full bloom; Fig. 70, 2 months; Fig. 71, 3 months; Fig. 72, 4 months; Fig. 73, 5 months; Fig. 74, 6 months; Fig. 75, 7 months; Fig. 76, 8 months; Fig. 77, 9 months. en, endocarp; emp, elongated mesocarp parenchyma; len, lignified endocarp.


68
69
70
71
72
73
74
75
76
77
emp
en
len
100 Microns

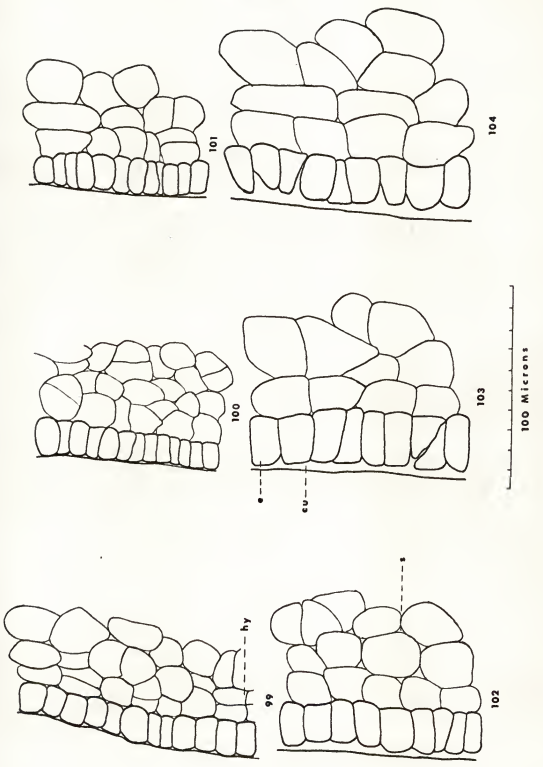
Figs. 78-84.--Cellular changes in epidermis and hypodermis of 'Waldin' avocado from full bloom to fruit maturity: Fig. 78, full bloom; Fig. 79, 1 month after full bloom; Fig. 80, 2 months; Fig. 81, 3 months; Fig. 82, 4 months, Fig. 85, 5 months; Fig. 84, 6 months. cu, cuticle; e, epidermis; hy, hypodermis.


cu
e
hy
78
79
80
81
82
83
84
100 Microns

Figs. 85-98.--Cellular changes in the mesocarp and endocarp of 'Waldin' avocado from full bloom to fruit maturity: Fig. 85, mesocarp cells at full bloom; Fig. 86, 1 month after full bloom; Fig. 87, 2 months; Fig. 88, 3 months; Fig. 89, 4 months; Fig. 90, 5 months; Fig. 91, 6 months; Fig. 92, endocarp cells at full bloom; Fig. 93, 1 month after full bloom; Fig. 94, 2 months; Fig. 95, 3 months; Fig. 96, 4 months; Fig. 97, 5 months; Fig. 98, 6 months. en, endocarp; emp, elongated mesocarp parenchyma cells; len, lignified endocarp.


85
86
87
88
89
90
91
92
93
94
95
96
emp
eh
97
len
98
100 Microns

Figs. 99-104.--Cellular changes in epidermis and hypodermis of 'Capac' avocado from full bloom to fruit maturity: Fig. 99, full bloom; Fig. 100, 1 month after full bloom; Fig. 101, 2 months; Fig. 102, 3 months; Fig. 103, 4 months; Fig. 104, 5 months. cu, cuticle; e, epidermis; hy, hypodermis; s, intercellular space.


99
hy
100
101
102
s
103
co
104
100 Microns

Figs. 105-116.--Cellular changes in the mesocarp and endocarp of 'Capac' avocado from full bloom to fruit maturity. Fig. 105, mesocarp cells at full bloom; Fig. 106, 1 month after full bloom; Fig. 107, 2 months; Fig. 108, 3 months; Fig. 109, 4 months; Fig. 110, 5 months; Fig. 111, endocarp cells at full bloom; Fig. 112, 1 month after full bloom; Fig. 113, 2 months, Fig. 114, 3 months; Fig. 115, 4 months; Fig. 116, 5 months. en, endocarp; emp, elongated mesocarp parenchyma cells; len, lignified endocarp.

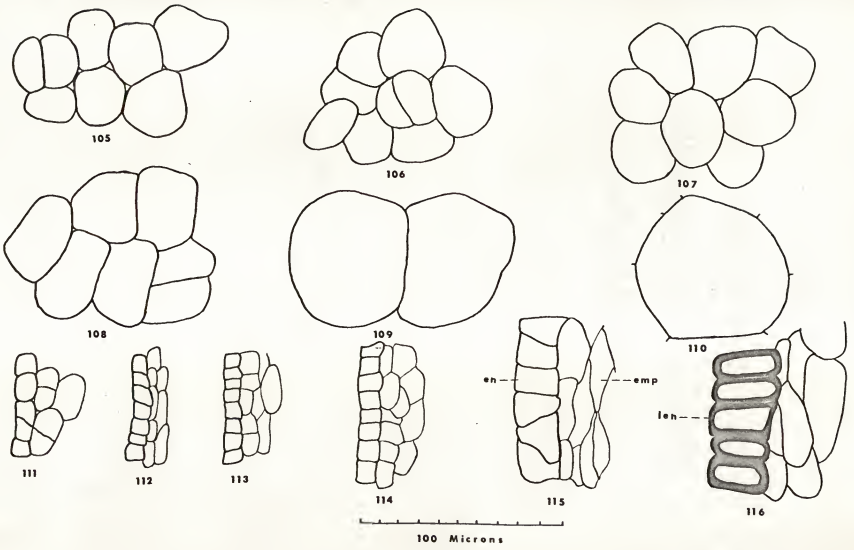
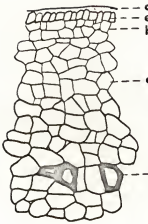

105
106
107
108
109
110
111
112
113
114
115
116
eh
emp
leh
100 Microns

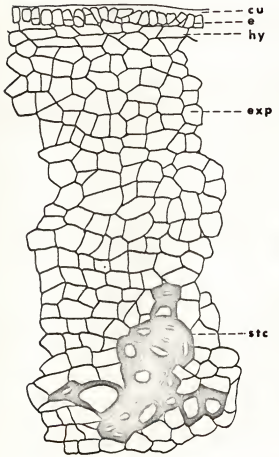
Fig. 117.--Cross section of 'Capac' exocarp showing a few isolated, relatively thin-walled stone cells. cu, cuticle; e, epidermis; hy, hypodermis; exp, exocarp parenchyma; st, stone cells.

Fig. 118.--Cross section of 'Waldin' exocarp showing a small cluster of stone cells. cu, cuticle; e, epidermis; hy, hypodermis; exp, exocarp parenchyma; stc, stone cell cluster.

Fig. 119.--Cross section of 'Taylor' exocarp showing a massive cluster of stone cells. cu, cuticle; e, epidermis; hy, hypodermis; exp, exocarp parenchyma; stc, stone cell cluster.




117




118

119

500 Microns

Cuticle.--At full bloom, the cuticle is visible in most places on the outer tangential walls of the epidermal layer of the 3 avocado varieties under study (Figs. 48,78, and 99). It is a very thin layer that stains light orange with Sudan IV in young fruits and dark orange in maturing fruits. Changes in cuticle thickness of the 3 avocado varieties studied are recorded in Tables 7,8, and 9.

Transverse and longitudinal sections of entire fruits show that the cuticle is continuous and covers the entire fruit surface, except over the stomata where it shades off to nothing. Two weeks after full bloom, the cuticle remains thin, measuring about 1.5 microns in thickness in 'Waldin' and 'Capac' and only about 1.0 micron in 'Taylor'.

One month after full bloom, cuticle thickness has increased a little. Longitudinal sections of entire fruits show that cuticle development is uneven. It is thicker at the apical end than at the basal portion. Where thick, the color reaction with Sudan IV is deep orange, as compared to the light orange color where this waxy layer is thin. The average thickness of the cuticle is now 2.0 microns in the 3 avocado varieties.

Six weeks after full bloom, the clearly visible cuticle has become fairly uniform in thickness over the whole fruit. The outer surface is smooth while the inner boundary is uneven, due to the slight depositions of this material between the radial walls of the epidermal cells. Measurements based on thickness above the outer tangential walls of the epidermis show that the cuticle on this date averages 3 microns thick in all 3 varieties. In the 'Taylor', where younger fruits are covered by numerous hairs, thick cutin deposits along hair bases ex-

tend down the radial walls of the epidermal layer.

Approximately 2 months after full bloom, the cuticle has grown to 4.0 microns thick in 'Taylor' and 'Capac' but only 3.5 microns in 'Waldin'. The outer surface of the cuticle remains smooth over the whole fruit (Fig. 120). Cutinization along the radial walls of the epidermal cells is more advanced at the apical end, where cutin deposits can be seen almost half-way along these walls.

Ten weeks after full bloom, the cuticle has grown to 5.0 microns in thickness in all 3 varieties. The slow deposition of cutin between cell walls of the epidermis continues, and in a few cases in 'Capac', cutin has actually reached the hypodermal layer. An important observavation on this stage of development is the scaling of the cuticle on the apical portions of 'Capac' and 'Waldin' fruits. These occasional scalings, however, are shallow and do not expose the epidermal cells.

Two weeks later,the cuticle of 'Taylor' has increased 0.5 micron in thickness but those of 'Waldin' and 'Capac' remain the same. However, the deposition of cutin along the radial walls of the epidermis continues and in a few cases, individual cells or pockets of 2 to 4 cells have been completely enclosed by a thin layer of cutin. The occasional shallow scaling of the cuticle is still confined to the apical end and the outer surface of the cuticle remains smooth in all other portions of the fruit. In a few instances, lenticel formations have caused the rupture of the epidermal layer and also of the cuticle, thereby breaking the continuous and even coating of this waxy substance all over the fruit.

Fourteen weeks after full bloom, the cuticle is still about 5.0 microns thick in both 'Waldin' and 'Capac', while in 'Taylor' it has

grown to 6.0 microns in thickness. Cutinization in 'Taylor' has been slow, but at this stage of development, it can now be observed going on all over the fruit. At the apex, where it started, cutin deposition has reached the outer hypodermal layer and in a few cases formed along the inner tangential walls of the epidermal cells.

Sixteen weeks after full bloom, the slow deposition of cutin has continued in 'Taylor' and 'Waldin' and the thick cutin is beginning to extend between hypodermal cell walls in 'Waldin' (Fig. 121). In 16-weeks-old 'Capac' fruits, cutin deposits have become very extensive along radial walls of the epidermis and on its inner tangential walls, except at the basal end of the fruits. In one instance, cutin deposits on the inner tangential walls of the epidermis stretched the entire length of 23 epidermal cells.

Measurements of cuticle thickness 18 weeks after full bloom show that the 3 varieties again have the same thickness, 6 microns. The outer surface of the cuticle remains even and smooth. Cutin depositions along the inner walls of the epidermis continue but it is only in 'Waldin' that cutinization has extended down to the hypodermal layer.

The cuticle of 'Capac' fruits at maturity is 7.0 microns in thickness. Cutinization of the inner walls of the epidermal cells is very profuse but there is no observable deposition of cutin along radial walls of the hypodermis. Cutinization in 20-weeks-old 'Taylor' fruits is similar to that of 'Capac'. No cutin deposit is ever found in the hypodermis, but the continuity of 'Taylor' cuticle has become broken in many places by lenticel formation. This is true for 'Waldin' also, but in a less degree. 'Waldin' cuticle remains at 6.0 microns in thickness while that

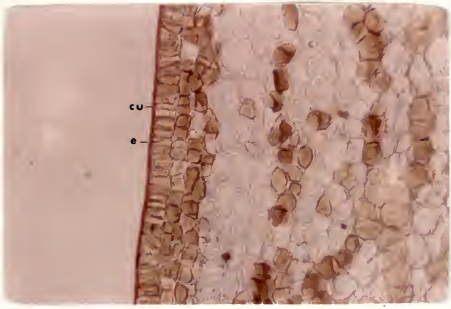



Fig. 120.--Longisection of young avocado fruit showing the smooth and thin cuticle. cu, cuticle; e, epidermis.

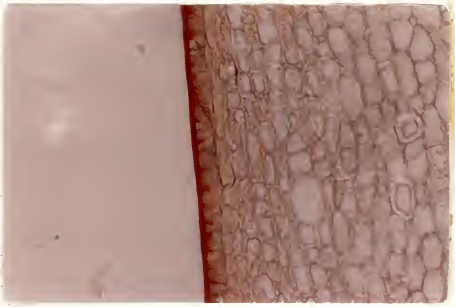


Fig. 121.--Longisection of maturing avocado fruit showing cutin deposits along radial walls of the epidermis.

of 'Taylor' has grown to 6.5.

Five months after full bloom and 2 weeks after acceptable maturity of 'Capac' fruits, the cuticle still measures 7.0 microns in thickness. The outer surface of the cuticle has remained smooth all throughout the life of the 'Capac' fruits except for shallow scalings at the apical ends and the occasional stomata and lenticels. Cutin deposits are limited to the epidermis, which is made up of a single layer of cells. The cuticle of 22-weeks-old 'Taylor' fruits also measures 7.0 microns thick. At maturity, the cuticle of 'Waldin' fruits is 6.0 microns thick.

Two weeks after 'Waldin' fruits reached commercial maturity, the cuticle has increased to 7.0 microns in thickness. The outer surface remains smooth and the slow penetration of cutin into the hypodermal layer has practically stopped. The cuticle of 'Taylor' fruits 24 weeks after full bloom is still 7.0 microns in thickness. Although cutin deposits on the inner walls of the epidermal cells have grown thicker, no cutinization can be observed along the tightly packed, radially flattened hypodermis.

In slides prepared from fruits of 'Waldin' collected 4 weeks after attainment of horticultural maturity, cuticle thickness above the outer tangential walls of the epidermis remains 7.0 microns in thickness. The occasional shallow scalings in the cuticle never extend down to the epidermis. Only where lenticels occur is the cuticle ruptured, exposing a few cells of the filling tissue. Fruits from this collection that were allowed to ripen before fixing did not show any difference in cuticle thickness and structure from those fruits fixed while still firm. The only observable change in 'Taylor' cuticle in the last 2 weeks is the increase in thickness to 8.0 microns.

On September 23, 1963, 28 weeks after full bloom of the 'Taylor' avocado, a few fissures appear in the cuticle. The continuous deposition of cutin within the epidermal layer has proceeded at a slow rate but the cuticle remains 8.0 microns in thickness. In another 2 weeks, many fissures have appeared in the cuticle. These fissures are shallow and do not run through the entire thickness of the cuticle, which at this stage is still 8.0 microns thick.

From October 7 to November 18, 36 weeks after full bloom and 2 weeks after 'Taylor' avocado fruits reached horticultural maturity, the only observable change in the cuticle is the stretching of the fissures that appeared earlier. No more new fissures have appeared and the breaks in the old ones have never reached the epidermal layer. A thin cuticular layer always remains covering the outer tangential walls of the epidermal cells. The cuticle has not increased in thickness anymore, but has remained at 8.0 microns since early September. As in 'Capac' variety of avocado, cutin depositions are limited to the epidermal walls of the fruits. The cuticle of fruits from the last collection that were allowed to ripen remained identical in all respects to those of freshly collected mature fruits.

Epidermis.--The epidermal layer of avocado fruits at full bloom is one cell layer thick. The epidermal cells are thin-walled and actively dividing. Several anticlinal cell divisions were observed in the 3 avocado varieties studied. The cell contents stain red with safranin-fast green (Fig. 122). Tests with ferrous sulphate (Johansen, 20) indicate that these cells contain tannin. The red epidermal layer is clearly distinguishable from the light green cells of the mesocarp tis-

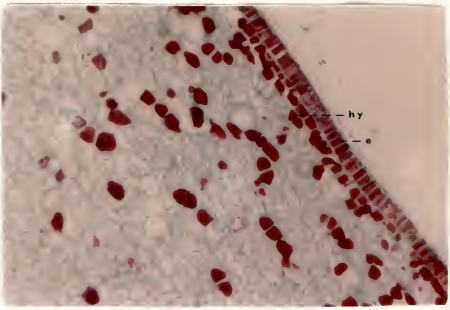



Fig. 122.--Shows the red-staining epidermis, hypodermis and a few exocarp parenchyma cells of young avocado fruit. Microchemical tests indicated the presence of tannin in these cells. e, epidermis; hy, hypodermis.

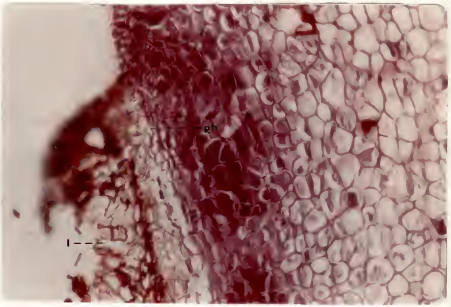



Fig. 123.--Shows a ruptured lenticel and the cork-producing phellogen. l, lenticel; ph, phellogen.

sue. The epidermal cells can also be distinguished from the subjacent cells by their shape. They are somewhat radially elongated while those of the hypodermis directly beneath them are radially flattened (Figs. 48,78 and 99). The few stomata observed are level with the surface of the epidermis, and are regular in morphology, consisting of a pore surrounded by 2 simple guard cells.

Measurements of cell-size and cell-dimension changes in the epidermis of the developing 'Taylor', 'Waldin', and 'Capac' avocado fruits are presented in Tables 7, 8, and 9.

Two weeks after full bloom, the epidermal cells at the basal end have become tightly packed and well-organized. They are brick-shaped and radially elongated. Those at the apical portion remain somewhat loosely arranged. The nuclei of these thin-walled and actively dividing cells are easily visible. Only anticlinal divisions, which are more common at the basal end, can be observed at this stage of fruit development. The epidermal layer has remained 1 cell layer thick. The basal portions of epidermal hairs are sometimes encountered but are sloughing off.

One month after full bloom, the epidermal cells have become more compactly arranged. They are now uniformly brick-shaped from the basal portion up to the apex of the young fruits. Practically every single cell in this layer is undergoing anticlinal division. Because no periclinal cell divisions occur, this layer has remained 1 cell thick. The radial diameter of these brick-shaped cells is now about twice the longitudinal and transverse-tangential diameters. The large, deeply stained nuclei of these actively dividing cells are clearly visible.

Development of the epidermal cells at the 6th week after full

bloom follows the same pattern as that of the previous collection. Anticlinal cell divisions go on very rapidly and the epidermal cells remain very tightly packed together. Although the young fruits of all 3 varieties have increased almost 7-fold in volume and weight in the last 2 weeks, the size of the epidermal cells has remained practically unchanged. This layer, at this age, must keep up with fruit growth mainly through cell multiplication.

Little change was found to take place in the avocado epidermal layer during the next 2 weeks. Anticlinal cell divisions continue at a rapid rate and cell dimensions remain almost stationary except for slight increments along the radial diameter. In 'Capac', the epidermis has developed some raised areas. These are due to lenticel formation beneath old stomata. The filling tissue that develops in these lenticels has forced the epidermal layer outward, but no ruptures are visible at this date. Tests with 50% chromic acid (20) show that the filling tissue is composed of suberized cells.

Ten weeks after full bloom, lenticel formation is also discernible in 'Taylor'. The surface of the rind has become conspicuously rough and "pebbled." When viewed in transverse or longitudinal sections, the epidermal layer can be seen to ride over these "pebbled" areas and maintain a continuous single layer of closely packed, radially elongated cells. Lenticels have increased in number in 'Capac' avocado. There has been very little increase in epidermal cell diameters but anticlinal cell divisions continue rapidly.

An interesting observation on the epidermis of the 3 avocado varieties 12 weeks after full bloom is the appearance of oblique cell divi-

sions. Instead of producing 2 regular and equal-sized daughter cells, the obliquely dividing mother cells produce 2 disparate daughter cells; in many cases, one of them is much smaller than the other. A few periclinal cell divisions are also observed in the apical portions of the developing fruits. Because of this unusual type of cell division, the epidermal layer at the apical end has become slightly disorganized. Numerous anticlinal cell divisions have proceeded normally in the mid and basal portions of the fruits. Because of rapid proliferation of the filling tissue of the young lenticels in 'Taylor' avocado, many ruptures of the epidermis of this variety are observed.

On the 14th week after full bloom, the oblique and periclinal cell divisions previously observed in the apical end can now be seen all over the fruit. However, there seems to be very little radial expansion among the daughter cells produced by the occasional periclinal divisions and the chips resulting from oblique cell divisions, thereby retaining the distinctness of the epidermal layer.

Sixteen weeks after full bloom, the close-knit organization of the epidermal layer has begun to break up. Periclinal divisions have become prevalent. The tabular epidermal cells seldom divide at the middle to produce sister cells of equal sizes. This, coupled with oblique cell divisions that result in the production of chips of many shapes and sizes, has caused a disorganized appearance of the epidermal layer. At the apex, where this is more common, 2 layers of epidermal cells may be seen in the 'Waldin' and 'Capac'; but in the rest of the fruit, the normal anticlinal cell divisions proceed as usual. On this date, the radial diameter of the normal cells has become more than twice the longitudinal

and transverse-tangential diameters.

Observations on the epidermal layer of materials collected 2 weeks later showed that normal anticlinal cell divisions have slowed down and that the individual epidermal cells are separated further from one another by extended cutin deposits. Although cutin has frequently cut off and surrounded either single cells or groups of cells, they evidently remain alive, because they continue dividing. Even though many adjacent cells may have divided once periclinally, no clear-cut second layer is formed because the cell plates of adjacent dividing cells seldom form at the same level. Towards the middle and basal portions of the fruit, the epidermal layer has remained essentially a single cell in thickness.

As 'Capac' fruits reach maturity, 20 weeks after full bloom, the protoplasmic cell contents have become less dense and in some cases disorganized. However, occasional dividing cells can still be observed, which means that some cells are still living, but they are becoming less active. Cross sections of the rough-skinned 'Taylor' show that the filling tissue of the numerous lenticels comes from a subepidermal phellogen. This phellogen produces large masses of cork cells which rupture the epidermis (Fig. 123).

Two weeks after 'Capac' fruits have reached maturity, the epidermal layer remains continuous over the fruit surface (Fig. 104), except for the few lenticels that have erupted and caused breaks in this layer. In the mature 'Waldin' fruits, the epidermal cells have become more disorganized, with the cells being further separated by extended cutin deposits and disoriented cell divisions. The majority of the lenticels of this variety, as well as of 'Capac', do not break through the epidermis.

Examination of the epidermis of 'Waldin' fruits collected 2 weeks and 4 weeks after reaching horticultural maturity reveals that the epidermal cells continue increasing slowly in size as long as the fruits remain attached to the trees. At these last stages of fruit development, the epidermal cells appear inactive but not dead, since dividing cells are observed, although very sparingly (Fig. 84). The rind of 24-weeks-old 'Taylor' fruits is becoming increasingly rough in texture due to the cork cells which form in the lenticels. Several lenticels sometimes coalesce to form large, corky patches.

From September 9 until the last collection on November 18, 1963, there was very little increase in size of 'Taylor' epidermal cells. Normal, anticlinal divisions continue but at a much slower rate. The more common type of cell division is the oblique one, which causes a disorganized condition of the epidermal layer. Rapid invasion of the cutin between the walls of the "chips" and other irregularly-shaped daughter cells results in separation of the cells of this layer. The epidermis, however, remains a distinct layer up to maturity, bound by the continuous and uniform cuticle on the outside and the tightly packed, radially flattened hypodermal cells directly beneath it (Fig. 57).

Hypodermis, Exocarp Parenchyma, and Sclerenchyma.--At full bloom, the hypodermis of avocado fruits is recognizable but still not well defined. Differentiation of the hypodermal cells at the stylar end is already completed. The cells in this region are radially flattened. This layer is mostly 1 cell thick but in some locations, products of periclinal cell divisions have started the formation of a second layer. These newly differentiated hypodermal cells take a red color with safranin-fast green. The cells become less elongated as the mid-section of the pistil

is approached, and towards the basal end, the cells directly subjacent to the tabular epidermal cells are regular in shape, stain light green, and do not differ from the cells of the mesocarp. The cells in this region are actively dividing in both tangential and radial planes, but at the stylar end, periclinal cell divisions predominate. Diameter measurements at the midsection of these pistils show that the cells are radially flattened. There are no sclereids at this early age nor is there any recognizable exocarp parenchyma in the 3 avocado varieties under study.

Two weeks after full bloom, all the hypodermal cells at the basal end of the developing fruits have divided once periclinally, making this layer 2 cells thick. Although elaboration of the cells in this layer is rapid in the apical portions, as seen in longitudinal sections of whole fruits, differentiation of hypodermal cells towards the basal end is slow. A single layer of radially flattened hypodermal cells is slowly being formed beyond the middle of the young fruits. The cell contents of the hypodermal layer stain red with safranin and microchemical tests indicate the presence of tannin in these cells (Fig. 122).

One month after full bloom, a well-defined hypodermal layer can finally be seen at the basal end of the developing fruits. At this stage of development, the hypodermis has already formed a continuous layer of 2 to 3 cells in thickness beneath the epidermis. An important observation at this stage of fruit development is the appearance of tannin-filled cells beneath the hypodermis and surrounded by green staining parenchyma cells (Fig. 124). They are located from 3 to 10 cell

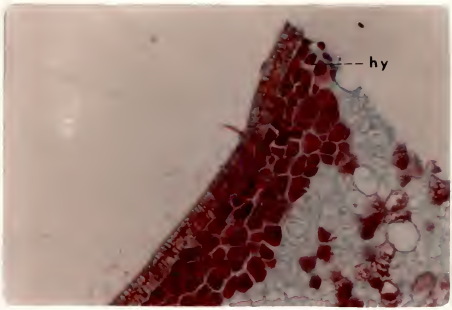



Fig. 124.--Longisection of an older avocado fruit showing a thicker hypodermis. hy, hypodermis.

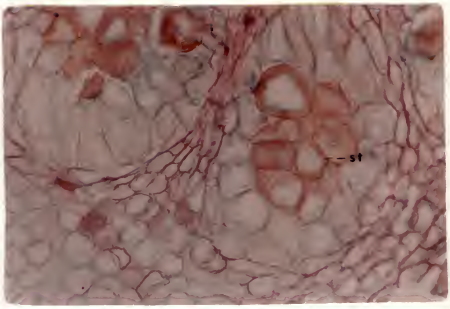



Fig. 125.--Illustrates the radiating pattern of newly formed sclereid initials that surround some earlier differentiated stone cells in the avocado exocarp. st, stone cell.

layers deep below the hypodermis and are more prevalent at the stylar end.

Two weeks after the hypodermis has become a continuous layer beneath the epidermis, the 2 to 3 cell layers making up this subepidermal tissue have assumed a more compact appearance. The cells have increased little in size but cell division is active. The parenchyma cells subjacent to the hypodermis are smaller and have thicker walls than those found deeper in the mesocarp tissue. The tannin-filled cells previously found mainly at the apical end of the fruits have by now appeared at the basal portion also.

In the hypodermis itself, little change can be observed 2 months after full bloom. Anticlinal divisions are more commonly seen in this layer at this stage of development. Periclinal divisions have slowed down, but in 'Waldin' fruits, the hypodermis has grown to 4 cell layers thick at the apical portion (Fig. 124). Some oblique cell divisions have taken place in the hypodermis which have resulted in small, irregularly-shaped intercellular spaces, but the largest intercellular spaces are found in areas where lenticels have formed below stomatal openings. The parenchyma cells directly beneath the hypodermal layer resemble those in the mesocarp but differ in having thicker walls and in containing tannins. In spite of their thicker walls and tannin content, these cells are active and carrying on cell division just like the parenchyma cells in the mesocarp. These tannin-filled cells have appeared as far as 30 cells deep into the mesocarp tissue at the apex and 18 cells deep in the equatorial section of the developing fruits. A significant change in 'Taylor' fruits is the appearance towards the apex of a few stone

cells in the zone dividing the exocarp parenchyma from the mesocarp parenchyma. The walls of these young stone cells stain light red with safranin. Upon the addition of phloroglucin and hydrochloric acid (40), the walls of these stone cells become red-violet in color, confirming their lignified condition.

In the next collection, two weeks later, few observable changes have taken place in the 'Waldin' and 'Capac' fruits, but in 'Taylor' some significant transitions have occurred. Many new lenticels have formed. Each pebbled area or protrusion on the 'Taylor' rind is the result of the formation of great quantities of cork cells in these lenticels. Below these loosely arranged cork cells is the lenticel phellogen composed of radially flattened cells. The phellogen cells bend slightly inward, forming a concave row of closely packed cells dividing the small, loose, suberized cells of the filling tissue from the well-arranged exocarp parenchyma beneath. A few more sclereids have been initiated in the apex of 'Taylor' fruits. In the course of this development, a parenchyma-like cell begins to enlarge and becomes distinct from its neighbors by its larger size. As it reaches full size, the cell is found to be occupied by a large central vacuole surrounded by a parietal layer of cytoplasm. After growth in cell diameter has ceased, the wall begins to thicken and the presence of numerous pits over its surface becomes evident. As the cells become older, lignification and thickening of the walls continue further.

Twelve weeks after full bloom, the hypodermal layer has become interrupted in many locations by the appearance of lenticels in the exocarp of the developing fruits. The almost isodiametric cells comprising

the filling tissue of these lenticels proliferate rapidly and disrupt the hypodermal layer. Later on, these cork cells push the epidermal layer outward, so that eventually it bursts open. The exocarp parenchyma now has much-thickened walls which are slightly suberized, as shown by chromic acid tests (20). Stone cells have appeared for the first time in the apex of 'Waldin' fruits, while those found in 'Taylor' have increased in number. The differentiation of new stone cells has occurred concentrically around some earlier formed ones. As the cluster of stone cells becomes bigger, the walls of the older ones in the center grow thicker.

The hypodermal layers of 14- and 16-weeks-old fruits have not changed much. They have increased little in size and have remained from 2 to 5 cells thick. However, the walls of the exocarp parenchyma cells beneath the hypodermis have thickened considerably. Wall thickening is not angular, but is even throughout. These cells are ascertained to be parenchyma when they fail to acquire a red coloration with ruthenium red dye (13). Another significant change is the proliferation of stone cells, especially in 'Taylor'. Figure 125 illustrates the radiating pattern of parenchyma cells that surround the clusters of stone cells, like those described by Sterling (60) in the pear fruit.

Eighteen weeks after full bloom, the first stone cells appear at the apical portion of 'Capac' fruits. They differentiate in a zone 13 to 18 cells below the epidermis and appear either singly or in groups of 2 to 4 cells. Their walls are relatively thin. In 18-months-old 'Waldin' fruits, the clusters of stone cells at the apical end of the fruits have grown much bigger, sometimes reaching 15 or 16 in number.

Stone cells have also appeared at the basal portion of 'Waldin' fruits at this time, but they are still relatively thin-walled and few (Fig. 118). In the woody exocarps of 'Taylor', proliferation of stone cells has been much faster. In certain locations, especially towards the apical end, the enlarging clusters of stone cells have almost coalesced, thereby forming a nearly continuous layer of sclerified cells.

At maturity of 'Capac', 20 weeks after full bloom, cell divisions in the hypodermis have slowed down, but increments in cell size continue. Many lenticels can be observed in 'Capac' exocarp but they are mainly the non-eruptive type. The stone cells in this variety remain few and thin-walled (Fig. 117). Examination of 'Waldin' materials of this age reveals the presence of a phellogen. In longitudinal sections, the phellogen cells appear rectangular and radially compressed. They differ from the parenchyma cells of the exocarp not only in shape but also in size, the phellogen cells being smaller. The clusters of stone cells in 'Waldin' continue enlarging as the fruits advance towards maturity. The differentiation of new stone cells is the main observable difference between 'Taylor' fruits of this collection and those collected 2 weeks earlier.

The development of 'Capac' hypodermis and exocarp parenchyma continues slowly. Measurements of cell dimensions, 2 weeks after maturity, indicate that the hypodermal cells continue to grow. Although cell division in this layer and in the exocarp parenchyma has slowed down considerably, a few dividing cells can still be observed, but stone cells never proliferate in this variety; they remain few and in many cases appear singly. This is in strong contrast with those of 'Taylor', which by now have proliferated considerably. The exocarp parenchyma in these

3 varieties at this date shows that most of its cells have tannin by their color reaction with safranin.

On August 26, 1963, 2 weeks after 'Waldin' fruits reached commercial picking, mitoses in the hypodermis and exocarp parenchyma have slowed down. Depositions of suberin along the walls of the above-mentioned tissues have grown thicker. Differentiation of new stone cells has also slowed down, not only in this variety but also in 'Taylor'. The changes going on in the stone cells at this time seem to be mainly wall thickening.

On September 9, 1963 the 'Waldin' and 'Taylor' fruits were 26 weeks old and 'Waldin' fruits were 4 weeks past initial commercial maturity. Measurements of cells in the hypodermal layer indicate that this tissue continues growing as long as the fruits remain on the tree. Studies on 'Waldin' fruits that were allowed to ripen before sectioning showed no difference in the make-up of the hypodermal layer.

Examinations of 'Taylor' specimens collected 28 weeks and 30 weeks after full bloom reveal that in this late-maturing variety, cell division in the hypodermis and mesocarp parenchyma goes on at a normal rate much longer than in the earlier-maturing varieties. Slow increments in cell size continue in these tissues, which have remained well-organized. A few stone cells are still being formed while the walls of the older ones continue thickening.

From October 21 until fruit maturity on November 18, 1963, the activity of the cells in the hypodermis and exocarp parenchyma slows down. At maturity, the hypodermis remains a well-organized layer of radially flattened cells ranging from 2 to 6 layers thick. This layer

is sometimes disrupted by lenticels. The hypodermal cells and the exocarp parenchyma continue their slow growth as long as the fruits remain attached to the trees. The walls of the stone cells have thickened considerably since their first appearance. These mature sclereids are composed almost entirely of the cell walls. The lumens are reduced to a small spherical or linear space in the center of the cell, and the bulk of the cell volume is made up of the centripetally deposited cell wall (Fig. 126). The stone cell clusters have also grown much bigger and coalesced, and have now formed an almost continuous zone of sclerified cells (Fig. 119).

Mesocarp

The cellular structure of the mesocarp parenchyma at full bloom is quite uniform (Figs. 58,85 and 105). These thin-walled cells are actively dividing in various random planes. Intercellular spaces are very minute and few, but many small branches of young vascular bundles are found among these cells. The xylem and phloem elements stain deep red with safranin-fast green, while the cell walls and the cytoplasm of the mesocarp parenchyma stain light green (Fig. 127). The mesocarp parenchyma cells are much larger than the cells in the epidermis and hypodermis (Tables 7,8 and 9).

The oil-bearing idioblasts reported by various earlier workers are found all over the pistils of the 3 avocado varieties studied, including the fleshy portions of the style. They appear singly and are easily distinguished by their much larger size and thicker walls (Fig. 127). The average radial diameter of these specialized oil-bearing cells at full bloom ranges from 43 microns in 'Taylor' to 54 microns in 'Capac'.

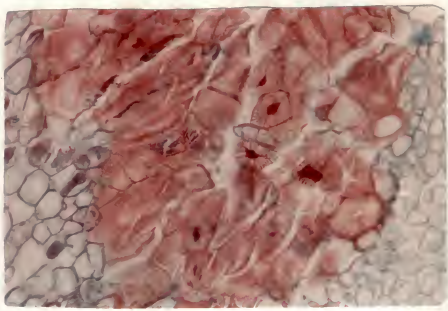


Fig. 126.--Shows a cluster of mature sclereids with thick secondary walls and much reduced lumens.

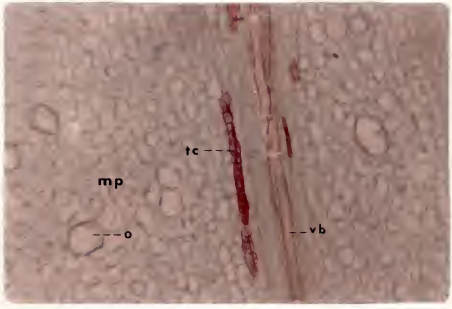



Fig. 127.--Longisection of avocado mesocarp showing the red-staining, lignified vascular bundles and a row of tannin-filled cells close to the vascular tissue. The walls of the mesocarp parenchyma cells and the large oil idioblasts stain light green. vb, vascular bundles; tc, tannin-filled cells; mp, mesocarp parenchyma; o, oil idioblast.

The bulk of the avocado mesocarp is composed of parenchyma cells that are quite uniform in shape and size. Because of the simple nature of the changes in this tissue during its development from the flower stage to the ripe fruit, the observations are presented, not on the bi-weekly basis that has been followed in the other tissue systems of this fruit, but rather on the basis of general changes in cell shape, cell size, and cell division as a whole.

Studies on the mesocarp of the young fruits disclose that the whole area is permeated with vascular tissue. The mesocarp parenchyma cells that lie along these vascular bundles are tangentially elongated. At full bloom, the mesocarp is only about 12 to 15 cells thick. For this reason, most of the parenchyma cells appeared slightly flattened along their radial diameter. The further the cells are located from the vascular tissue, the more isodiametric they become. As the fruits grow older and the flesh becomes thicker, the mesocarp parenchyma cells assume a more regular shape, and at maturity the cells are essentially isodiametric.

Cells close to the exocarp are usually slightly elongated in a tangential direction. These cells merge imperceptably with the isodiametric cells that spread almost throughout the thickness of the mesocarp. As the endocarp is approached, the mesocarp cells again become smaller and radially flattened. This observation is true for young fruits as well as mature fruits.

Changes in cell size are more evident than changes in shape. The bi-weekly increments in longitudinal, radial, and transverse-tangential diameters of the mesocarp parenchyma cells of 'Taylor', 'Waldin'

and 'Capac' avocado are presented in Tables 7, 8, and 9. By plotting the average radial diameter of the mesocarp parenchyma cells against age of the fruit (Fig. 128), it is seen that growth of the cells does not proceed at a constant rate. It follows a characteristic course of increase, first increasing slowly, then at a more rapid rate, and finally slowing down again when maturity is approached. The curve of the early variety 'Capac' was more steep than that of the late-maturing 'Taylor'. On the other hand, the slope of the growth curve of 'Taylor' was slightly more moderate than that of the mid-season 'Waldin'. In the late-maturing variety, the ultimate cell size was virtually attained several weeks before maturity. Thereafter, cell size increased little.

An interesting observation on the avocado mesocarp is the occurrence of cell division in this tissue even in maturing fruits. Because of this continued cell division, the number of layers of cells traversing the growing avocado mesocarp also increases constantly (see Tables 10, 11 and 12). When number of cells traversing the mesocarp (Table 10) and mesocarp thickness (Table 13) are plotted against age of avocado fruit (Fig. 129), it shows that increases in thickness of the mesocarp is not entirely the result of cell expansion but is also due to an increase in number of cells. For instance, the 3-mm thickness of the mesocarp of the young, 6-weeks-old 'Taylor' fruit is composed of a layer of approximately 87 cells. A mature, 34-weeks-old 'Taylor' fruit with a mesocarp thickness of 14.7 mm has 223 cells. This is more than twice as many cells along the radius. The average radial diameter of the mesocarp cells of the young fruit is about 35 microns and the average radial diameter of the mature fruit is about 66 microns. This

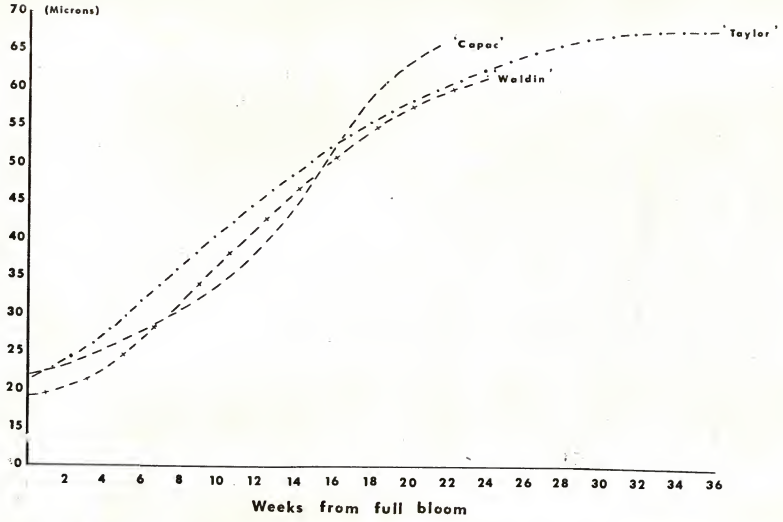



Fig. 128.--Average radial diameter of mesocarp parenchyma cells plotted against age of fruit.

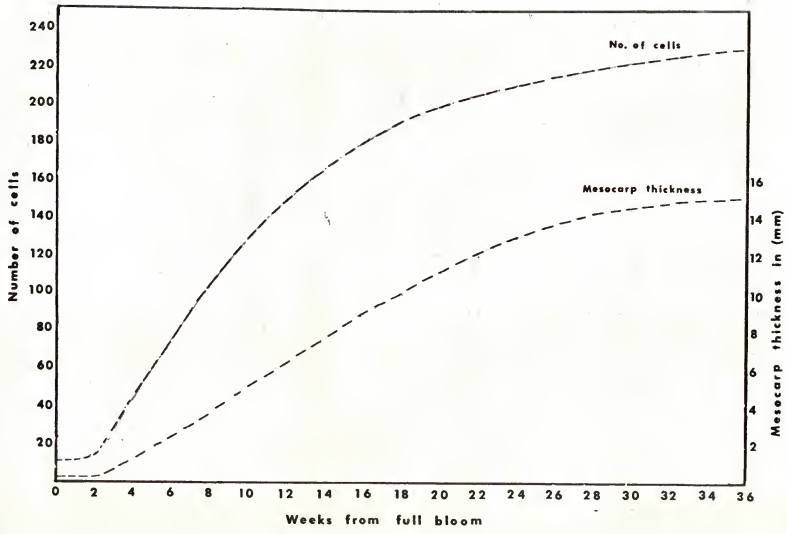

No. of cells
Mesocarp thickness
Number of cells
Mesocarp thickness in (mm)
Weeks from full bloom
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
20 40 60 80 100 120 140 160 180 200 220 240
2 4 6 8 10 12 14 16

Fig. 129.--Cell number and mesocarp thickness plotted against age of 'Taylor' avocado fruits.

confirms that mesocarp thickness, and hence growth of avocado fruits, in general results from both cell enlargement and cell multiplication which persists throughout the life of the fruits.

TABLE 13

AVERAGE INCREMENTS OF MESOCARP THICKNESS FROM FULL BLOOM TO MATURITY

| Weeks from full bloom | 'Capac' (mm) | 'Waldin' (mm) | 'Taylor' (mm) |
|---|---|---|---|
| 0 | 0.330 | 0.240 | 0.308 |
| 2 | 0.384 | 0.294 | 0.368 |
| 4 | 0.952 | 1.242 | 1.260 |
| 6 | 1.820 | 2.808 | 3.045 |
| 8 | 2.100 | 5.110 | 4.104 |
| 10 | 2.856 | 6.006 | 4.961 |
| 12 | 3.293 | 6.804 | 6.556 |
| 14 | 4.756 | 7.920 | 6.975 |
| 16 | 7.261 | 9.024 | 9.129 |
| 18 | 10.395 | 11.275 | 10.070 |
| 20 | 12.480 | 12.744 | 11.514 |
| 22 | 12.600 | 13.920 | 11.977 |
| 24 | | 14.760 | 12.915 |
| 26 | | 16.348 | 13.293 |
| 28 | | | 14.124 |
| 30 | | | 14.322 |
| 32 | | | 14.586 |
| 34 | | | 14.718 |
| 36 | | | 15.477 |

Cell size in the 3 varieties was essentially the same at full bloom and the ultimate cell diameter at maturity remained almost identical (Fig. 128 and Tables 7,8, and 9), but the size of the fruits varied considerably. The volume of 'Capac' fruits at maturity averaged only 204 cc while those of 'Taylor' and 'Waldin' were 425 and 531 cc, respectively (Tables 3 and 5). Since the size of the cells in these 3 varieties is es-

sentially the same at maturity, the differences in fruit size can only be attributed to differences in cell number. Furthermore, since it has been established that cell division goes on in the fruits of these varieties until maturity, the problem is not the duration of cell multiplication but its rate. An analysis of the whole developmental process with particular reference to the relative rates of cell division is presented in Table 14 and Figure 130. The increase in the number of layers of cells traversing the mesocarp of growing avocado fruits can only be attributed to cell division. When the percentage increase in number of cell layers over the number at the last previous date is plotted against time, the rate of increase (Fig. 130) clearly shows that cell division goes on very rapidly during the early part of fruit development. The rapid rate of cell division during the first 4 weeks of 'Waldin' fruits resulted in great increase in number of cells. This rate was 1.54 times faster than the rate of the small-fruited 'Capac'. Differences in size of fruits of the 3 varieties studied were due primarily to differences in rate of cell division and the resultant number of their constituent cells.

Observations on fully ripened fruits revealed few distinguishable cellular changes in the exocarp and endocarp tissues as compared to those of firm-mature fruits. But in the mesocarp, the very thin cell walls became undulate, and in many cases the walls began to break down. This cellular collapse was accompanied by an almost complete loss of stainability of the cell walls.

The oil-bearing idioblasts which occupy only 2% of the mesocarp tissue (10) were not studied in detail. It was noted, however, that the number and size of these idioblasts increased as the fruits grew older.

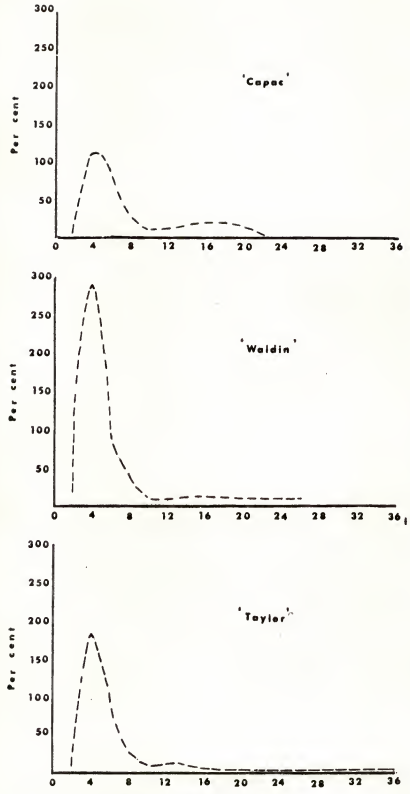



Fig. 130.--Rate of increase in number of layers of mesocarp parenchyma cells as a percentage of previous number of layers.

TABLE 14

RATE OF INCREASE IN NUMBER OF LAYERS OF MESOCARP PARENCHYMA CELLS AS A PERCENTAGE OF PREVIOUS NUMBER OF LAYERS

| Weeks from full bloom | Increments as a percentage of previous number of cell layers | | |
|---|---|---|---|
| | 'Capac' % | 'Waldin' % | 'Taylor' % |
| 2 | 6.6 | 16.6 | 14.2 |
| 4 | 112.5 | 285.7 | 181.2 |
| 6 | 91.1 | 92.5 | 93.3 |
| 8 | 7.6 | 40.3 | 24.1 |
| 10 | 20.0 | 5.4 | 12.0 |
| 12 | 5.9 | 5.1 | 23.1 |
| 14 | 30.3 | 11.1 | 4.0 |
| 16 | 18.1 | 6.6 | 15.4 |
| 18 | 20.4 | 6.7 | 6.1 |
| 20 | 16.3 | 5.3 | 6.3 |
| 22 | 4.1 | 7.4 | 0.4 |
| 24 | | 6.0 | 0.9 |
| 26 | | 8.9 | 2.9 |
| 28 | | | 1.4 |
| 30 | | | 1.4 |
| 32 | | | 1.8 |
| 34 | | | 0.9 |
| 36 | | | 3.5 |

These specialized oil-containing cells seemed to be differentiated from ordinary parenchyma cells. As was reported by Scott and co-workers (51), the differentiating idioblasts are already marked by the refringence of their contents. Mature idioblasts are filled with a large drop of oil. The size of these idioblasts in ripe fruits ranged from 80 to 100 microns. The presence of lignin, reported by earlier workers (10,51), was never observed in the thickened walls of these large, oil-bearing cells.

Another observation in the developing mesocarp tissue was the ap-

pearance of tannin-filled cells in the later stages of fruit development. These cells were limited to regions close to vascular bundles and were often arranged in strands several cells long and from 1 to 4 cells thick (Fig. 127).

Endocarp

The endocarp, the inner layer of the avocado pericarp, consists of a single row of cells. At full bloom, when the developing seed has not yet occupied the fruit cavity, the endocarp layer can be observed as a single row of nearly isodiametric cells (Fig. 131). At this stage, they are the smallest cells in the pericarp and are parenchymatous in nature. Their thin walls stain light green with safranin-fast green. Anticlinal divisions are actively going on in this layer. Adjoining the endocarp are a few rows of tangentially elongated mesocarp parenchyma cells.

From full bloom until close to horticultural maturity developmental changes in the endocarp are very slow and gradual. The cells remain parenchymatous, increase slowly in size, and undergo anticlinal divisions actively. The layer remains 1 cell in thickness and the cell walls become slightly thicker. This layer eventually comes in contact with the outermost layer of cells of the greatly enlarged seed. When the fruits are almost mature, anticlinal cell divisions slow down considerably and the cells start to enlarge at a faster rate. The cells in 'Taylor' and 'Capac' become radially elongated while those of 'Waldin' remain almost isodiametric (Figs. 77, 116 and 98).

When 'Capac' and 'Waldin' fruits reach maturity, their endocarp cells have become lignified. The thick walls stain red with safranin, and the phloroglucin-HCl test of Sass (40) confirms the presence of lignin in

Fig. 131.--Longisection of young fruit showing the endocarp as the innermost layer of the avocado pericarp. It consists of a single row of nearly isodiametric parenchyma cells. The seed is surrounded by a row of large, tannin-filled cells. e, epidermis; mp, mesocarp parenchyma; en, endocarp; t, tannin-filled cells; s, seed.

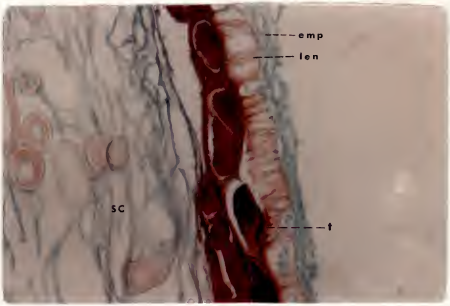



Fig. 132.--Longisection of mature avocado fruit tissue showing the lignified endocarp and a few rows of elongated mesocarp parenchyma cells adhering to the tannin-filled cells of the seed coat. emp, elongated mesocarp parenchyma; len, lignified endocarp; t, tannin-filled cells of seed coat; sc, seed coat parenchyma.

these walls. The walls of the elongated parenchyma cells adjoining the endocarp have become very thin and are starting to disintegrate. In ripe avocado fruits, the endocarp often adheres to the seed coat when the seed is removed and it is in adjacent rows of tangentially-elongated, thin-walled mesocarp parenchyma cells that tearing apart takes place.

Lignification of the endocarp cells is more gradual in the late-maturing 'Taylor' fruits. It is first observed when the fruits are 28 weeks old (Fig. 75). In subsequent collections, more cells become lignified. As in the differentiation of sclereids in the exocarp, the endocarp cells first enlarge, then large vacuoles appear, and finally the walls begin to thicken. But instead of the radiating pattern of sclereid differentiation, stone cells in the endocarp develop along the single row of cells, starting from older, lignified ones and extending in both directions. At maturity, every cell in the endocarp is lignified (Fig. 132).

Seed

The seed of the avocado consists of the embryo, with its large and fleshy cotyledons, and 2 adherent seed coats. These two are so closely conjoined that it is impossible to be sure which cells belong to which coat, and so the 2 coats are treated here as a single tissue. The outermost layer of the seed coats is composed of very large cells which are filled with tannin (Fig. 132). Underneath these are several layers of small parenchyma cells and a few strands of tannin-filled cells that lie along the vascular bundles permeating the seed coats. In mature fruits, several stone cells are differentiated within this parenchyma tissue. The cotyledons are formed of parenchyma cells and occasional strands of phloem and xylem elements. Starch grains are found in great abundance in the parenchyma cells of mature seeds. The size of these storage parenchyma cells of the cotyledons is almost the same in the 3 varieties of avocado studied.

## DISCUSSION

The term "growth" has been variously defined and Hammet includes in it not only volume increase but also the accompanying phenomena of progressive differentiation. Sinnott, in discussing plant morphogenesis, considers the permanent increase in volume as the most important aspect of growth (55). A plant increases in height mainly through the activity of meristems at the tip of each shoot. Increase in thickness is primarily through the activity of a lateral meristem, the vascular cambium (11). These meristems are rather sharply localized. In organs which have a limited or determinate growth, such as fruits, the meristems are usually not localized but are diffused, so that growth is not at any particular point but takes place almost throughout the whole fruit. The size that a plant organ attains at maturity is intimately related to growth, and Sinnott (55) says that "differences in ultimate size may be due to differences in rate or in duration of growth or in both."

The gross development of the avocado fruit follows the normal curve. The latter portion of the growth curves in early-maturing varieties is generally steep and the fruits are still increasing rapidly in size at harvest time, while in the late-maturing varieties, the growth curves are moderate and increments in size have slowed down considerably long before acceptable commercial maturity is attained. The fruits of both summer and winter varieties will continue growing as long as they remain attached to the trees, and fruits picked early in the harvesting period are generally smaller than those picked later in the season. While fruits of

a variety follow the same rate of growth, fruits belonging to different varieties often vary considerably in their growth rates. In the case of 'Waldin' and 'Taylor', which came to full bloom at the same time, their fruits were almost identical in size during the first 6 weeks. In subsequent collections, the growth curves showed that 'Waldin' had started to grow much more rapidly than 'Taylor'. This steady and rapid growth in 'Waldin' is reflected in the almost straight rise of the latter portion of its growth curve. By July 15, 1963, 18 weeks after full bloom, 'Waldin' fruits were more than twice as large as 'Taylor' fruits. Four weeks later, 'Waldin' fruits reached commercial maturity while those of 'Taylor' were still immature. Although the rate of growth in 'Taylor' was slow, it continued for a much longer time, and when the fruits first reached commercial harvesting on November 4, 1963, they were as large as the earliest harvested 'Waldin' fruits. These 2 varieties perfectly depict the influence of rate and duration of growth on the final size of a plant organ.

The gross-size increments in fruits during development are the result of cell divisions and cell enlargements in varying proportions in different tissues at different times (67). Studies in the developmental anatomy of the apple (4,62 and 68), peach (1), sour cherry (67), date (28), strawberry (18), and such vegetables as tomatoes (19) and cucurbits (53) indicate a period of rapid and extensive cell division following full bloom and extending for a few weeks after pollination. After this short period of cell division ceases, the fruits increase in size only from cell enlargement. The avocado is unique in that cell-division activity is not restricted to the initial period of fruit growth but also goes on

even in maturing fruits, as reported by Schroeder (45) and confirmed in this work.

The problem of the relation of cell number and cell size to organ size is not a simple one. According to Sinnott (55), Sachs and Amelung in 1893 presented evidence that size differences in plant organs result primarily from differences in the number rather than in the size of their constituent cells. More recently, studies in tomatoes (19) and gourds (53) have shown that the periods of cell multiplication and cell enlargement are both longer in the large fruits than in the smaller ones, so that the greater size of the former is due to both more and larger cells.

In avocado fruits, whether large or small, the ultimate cell size of the mesocarp parenchyma at maturity is essentially the same, but mature fruits differ considerably in size, sometimes as much as 160 per cent. These large differences in fruit size could only be related to the number rather than the size of the constituent cells. When the relative rates of cell divisions are analyzed, they demonstrate that during the early life of the fruits the process of cell division is very intense and the rate of cell multiplication in the large-fruited 'Taylor' is much faster than the rate of the small-fruited 'Capac'. The large and early-maturing fruits of 'Waldin' have a still faster rate of cell division than those of the slow-growing 'Taylor'.

The fact that cell divisions continue in maturing avocado fruits may explain in part why the fruits do not soften while still firmly attached to the tree (47). Perhaps the avocado fruit remains in a juvenile physiological state throughout its life on the tree primarily as the re-

sult of the continuous cell divisions which constantly contribute young cells.

There are evidently many anatomical similarities between fruits of different avocado races. The mesocarps of the 3 varieties studied exhibited a cellular architecture of considerable uniformity. Cummings and Schroeder (10) reported that the flesh of 'Fuerte', 'Nabal', 'Carlsbad', 'Nimlioh', 'Leonard', and 'Leucadia' avocado varieties also exhibited a cellular structure that was regular and uniform. The endocarp is also very similar in all varieties and is composed of a single layer of deeply pitted sclerenchyma cells. The study of the whole developmental process revealed that the endocarp cells start as a single layer of small parenchymatous cells that divide only anticlinally. As the seed increases in size and occupies the whole cavity, this layer comes in contact with the very large, tannin-filled cells of the outer seed coat. Continued growth of the seed exerts pressure on this layer and the thin-walled mesocarp parenchyma cells beneath. These 5 to 6 rows of mesocarp parenchyma later become radially flattened. When fruit reaches maturity, the endocarp cells become lignified and often adhere to the seed, together with a few rows of elongated parenchyma cells, when the fruit is cut open. These observations are contrary to an earlier report which stated that the endocarp "consists of a few rows of parenchyma cells which are smaller and somewhat more flattened than the adjoining cells of the mesocarp" and that "the outer layer of the seed coat consists of one to five layers of stone cells which are irregular in shape with heavy, deeply pitted, lignified walls" (10).

Two other observations made in the course of this study are con-

trary to previous reports. Schroeder (42) has reported that the whole epidermal cell wall is impregnated with cutin, so that the cuticle is the wall proper. Outside of this, he found a 2-layered wax coating. In the present study, the epidermal walls were always found to be cellulose, with a clearly differentiated layer of cuticle overlying it which seems identical to Schroeder's supra-cuticular wax layer. Likewise, cutinization of the lateral epidermal walls was clearly in the form of a layer replacing the middle lamella, and not an impregnation of the cellulose wall by cutin.

Idioblast walls have been reported as lignified in 'Fuerte' avocado by Cummings and Schroeder (10), but in the 3 varieties in the present study, such lignification was never found at any stage of maturity.

The anatomical differences between varieties belonging to the 3 avocado races were found in their exocarps. Young fruits have numerous stomata distributed over almost the entire surface. As the fruits approach maturity, the stomata gradually function less efficiently. In the thick-skinned 'Taylor', the deterioration of the stoma is followed by extensive cork formation, as a result of which large lenticels are produced. In the thin-skinned 'Capac', the proliferation of the filling tissue of the lenticels was slow and the epidermis was seldom ruptured. Another point of difference is the amount of stone cells differentiated in the exocarp. In the smooth and paper-thin rind of 'Capac', the sclerenchyma consists of a few isolated, relatively thin-walled stone cells that appear very close to fruit maturity (Fig. 117). In the smooth but leathery rind of 'Waldin', the stone cells are initiated early and later develop strongly lignified walls. They appear in clusters which enlarge as more stone cells are differentiated in the developing fruits. At maturity, these clusters remain

separated by parenchyma (Fig. 118). The differentiation of stone cells also starts early in 'Taylor' fruits. During their long period of development, stone cells are continuously being differentiated and the clusters enlarge considerably. Before the fruits mature, the clusters of stone cells coalesce in many areas and form an almost continuous layer of sclerified cells (Fig. 119). This is in part responsible for the woody and brittle texture of the rind of 'Taylor'. These observations are in agreement with the findings of Hass (16) and Cummings and Schroeder (10) in their studies on fruits belonging to the 3 races of avocado.

In the formation of sclereid clusters in avocado rinds, it appears that a "homoiogenetic induction" is promoted, so that adjoining cells are stimulated to become sclereid initials. The cell divisions that occur concentrically around some earlier formed sclereids and the transformation of these newly formed cells to stone cells with thick secondary walls that are strongly lignified support the "homoiogenetic induction" theory of Bunning (60). These findings do not support Cave's view that a sclereid cluster is derived from divisions of a single mother cell (60).

## SUMMARY

1. The gross morphology and histology of the developing fruits of the avocado from full bloom throughout the growing season to maturity are included in this study. Six varieties -- 'Capac', 'Blake Seedling', 'Waldin', 'Ruehle', 'Lula', and 'Taylor' -- were employed in the study of gross morphological changes and the following features of the fruit were recorded: (a) volume, (b) weight, (c) length of long axis from base to apex, (d) diameter at its widest part, (e) pericarp thickness, and (f) length of seed. Histological studies were conducted on 3 varieties ('Capac', 'Waldin' and 'Taylor'), each representing one of the 3 recognized races of avocado, and included changes in (a) epidermal hairs, (b) cuticle, (c) epidermis, (d) hypodermis and related exocarp tissues, (e) mesocarp, and (f) endocarp.

2. The avocado fruit is a berry composed of a soft pericarp enclosing a single large seed. The pericarp consists of 3 layers: the exocarp, which comprises the skin or rind; the fleshy mesocarp, which is the edible portion of the fruit; and an inner layer, the endocarp. The exocarp is made up of epidermal, parenchyma, and sclerenchyma tissues and is the layer which is removed when the fruit is peeled. The mesocarp consists of a mass of nearly homogeneous parenchyma cells with scattered oil idioblasts. Oil is abundant in the cytoplasm of the parenchyma cell and also fills the idioblasts. The mesocarp is permeated by vascular bundles which give rise to the "fibers" of the fruit. The endocarp consists of a single row of cells which become lignified at fruit maturity. Two papery seed coats surround the starchy embryo.

3. Growth of the avocado fruit follows the normal curve. In the early-maturing varieties, the rate of growth is fast and the growth curve is steep. The rate of growth in the late-maturing varieties is slow, resulting in a moderate slope of the growth curve. The ultimate size of the fruit is dependent upon both rate and duration of growth.

4. Increments in size of fruits are the result of cell multiplication and cell enlargement. Differences in fruit size of the different varieties, however, are related to cell number rather than cell size, because at maturity the size of the cells is essentially the same in both large-fruited and small-fruited cultivars.

5. The avocado fruit differs in its method of development from most other fruits which have been studied. In most fruits, the latter part of their development consists in enlargement of the previously formed cells. In the avocado, cell-divisions continue to increase the cell number within the tissues at all stages, even after horticultural maturity is attained.

6. The rate of cell division is very rapid during early fruit life. Differences in fruit size of varieties that mature at the same time result primarily from differences in rate of cell multiplication during the first 6 weeks of fruit development. The large-fruited 'Waldin', which matured only 2 weeks after 'Capac', exhibited a much higher rate of cell division than the latter, a small-fruited variety. A relatively slow rate of cell multiplication in the late-maturing varieties is compensated by the longer period during which mitoses can occur.

7. The main differences between the smooth, thin-skinned and the rough, thick-skinned varieties are in the extent of lenticel formation and sclerification in the exocarp. In the smooth-skinned varieties, only a

small number of cork cells are formed in the lenticels, which seldom break through the epidermis. In the rough-skinned varieties, large masses of cork cells are formed by a phellogen, causing the epidermis to erupt. Several lenticels often coalesce to form large, rough, corky patches. In the case of the thick-skinned varieties, part of the roughness in texture is the result of a massive stony layer that forms beneath the hypodermis. Only a few scattered stone cells are found in varieties with thin skins.

8. The avocado cuticle is a thin but clearly defined waxy layer on the outer wall of the epidermal cells.

9. The endocarp consists of a single row of cells with lignified walls constituting the inner layer of the pericarp.

10. The flesh of all varieties studied exhibited a very uniform cellular structure.

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Ramon V. Valmayor was born on December 17, 1931, in San Carlos, Negros Occidental, Philippines. He received his elementary and secondary education in that city. In June, 1951, he was awarded an entrance scholarship by the College of Agriculture, University of the Philippines. Soon after graduation in 1955, he joined the staff of the Division of Horticulture of the University of the Philippines.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 8, 1964

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